

Help, not sanctions, for North Korea

The prospect that North Korea will talk about its nuclear ambitions is the best outcome possible. The need is for a package of assistance to wean it (and others) away from bombs.

QUITE what will happen at the meeting now planned at Geneva between the United States and North Korea is anybody's guess, but the mere fact that there will be talks is a great deal better than the threat that North Korea might be subjected to sanctions agreed and imposed by the United Nations. At issue is North Korea's unwillingness to comply with the strict requirements of its safeguards agreement with the International Atomic Energy Agency (IAEA), necessary because of its membership of the Non-Proliferation Treaty (NPT). It is agreed that North Korea declined to let IAEA inspectors superintend the discharge of fuel from a graphite-moderated natural-uranium reactor at the end of May. The isotopic composition of the plutonium would have allowed guesses at its intended use: for making bombs or for some other purpose? That refusal is a plain violation of the IAEA agreement. But that, by itself, does not justify sanctions against a country already on its economic knees.

On the worst interpretation, North Korea cannot now have enough plutonium for more than one or two nuclear weapons. The plutonium in the fuel discharged in May could be enough for another handful, but even that is not clear. And although experienced nuclear-bomb makers may be able to design new versions with the assistance of computers, a newcomer to the business would not base a daredevil foreign policy on one or two untested bombs. Whatever may have happened, North Korea as it is is not a substantial nuclear threat to anybody. The goal must be to keep it that way.

How can that be done? It will not have been overlooked in North Korea that the nuclear powers (especially the United States) have established a number of awkward precedents recently. The idea that the West should help the Soviet Union with the cost of dismantling its nuclear weapons has been much talked of. More recently, there has also been talk of compensating the Ukraine for returning its nuclear weapons to Russia. That is *realpolitik* at its most real; it may also be called blackmail. Yet North Korea could well be looking for something tangible in return for giving up its flirtation with plutonium. What price would it be worth paying? And how would it then be possible to prevent other hitherto pacific signatories of the NPT from seeking to be bought off for not making plutonium?

The answer must surely lie in the NPT itself, which is essentially a pact between the nuclear and the non-nuclear powers. The former undertook in 1970 that they would provide the latter with technical assistance in the exploitation of civil nuclear power in return for an undertaking never

to make weapons (and to accept IAEA safeguards as an assurance of that). In reality, the nuclear powers' promise of technical assistance has not been worth much; the prospect that nuclear power stations would quickly proliferate has been disappointed by events. And the years immediately ahead do not look particularly hopeful either. So there is only a thin prospect that the non-nuclear signatories will in future benefit from the bargain that they struck.

That is why the best kind of price to pay at Geneva for North Korea's compliance with the NPT should be the promise of some other technical assistance than that promised by Article IV of the NPT. What form might that take? Isolation, not merely geographical, but economic, cultural and political, is North Korea's most serious handicap. And technically trained people are the best way of improving a country's economic and industrial performance. So why should there not be a programme of technical assistance in higher education aimed at improving the economic condition of North Korea? That might especially appeal to Japan's new prime minister, whose Socialist party is almost alone in its sympathy for North Korea. Of course, similar arrangements would have to be offered to other intermediate signatories of the NPT, but that device would at least provide the NPT with a stability it lacks at present. □

No news on Sunday

Nature will give up monitoring the coverage of AIDS by the *Sunday Times* in the hope that the wind has changed.

IN the week in which it emerged that AIDS, rather than violence with knives and guns, is now the chief cause of death among young men in Washington DC, and barely a month after providing Dr Kary Mullis with a public platform ineffectually to support the Duesberg view of the causation of AIDS (see *Nature* 369, 434; 1994), the London *Sunday Times* appears to have moderated its campaign on behalf of the belief that HIV is not the prime cause of AIDS.

The new developments are that Mr Andrew Neil, editor of the newspaper, has been seconded to Fox Television in the United States, and that Mr Neville Hodgkinson, the author of most of the articles in the past two years that have argued that HIV may be an irrelevance, has also left. Although the acting editor, Mr John Witherow, has defended the newspaper's previous coverage and even now insists that "the orthodoxy



that HIV causes AIDS does not match the facts", it is improbable that even if he wished, he could find anybody willing to follow in Hodgkinson's footsteps. Bluster apart, the *Sunday Times's* campaign on behalf of Duesberg is dead. For that reason, and until further notice, *Nature* will stop reporting on the newspaper's treatment of AIDS, thereby gaining valuable space and relieving readers of a degree of boredom.

It remains a matter of great interest how an aberration of this kind should have come about. The *Sunday Times* has from time to time been distinguished. Like the other British Sunday newspapers, it is a child of the British circumstance that most daily newspapers publish only on weekdays. When the present pattern emerged a century ago, there was rarely news to print on Sundays. Then various entrepreneurs discovered that even those who went to church welcomed something to read as well. Now the market is even bigger. But, except for the occasional disaster or political coup on a Friday evening or Saturday morning, there is still no news in the ordinary sense. So what can a Sunday newspaper do?

There have been several solutions. Some British Sundays fill their pages with accounts of small-time sex scandals, others go in for literary and other criticism and accounts of travel to places their readers are unlikely ever to visit. News is simulated by political commentary, but there are limits to what that can do. Then in the 1960s, Mr Harold Evans, newly appointed as the editor of the *Sunday Times*, hit on the excellent idea of introducing British newspaper readers to investigative reporting, the unravelling of public scandals by means of careful (and expensive) journalism. Among Evans's successes was the revelation that the British manufacturers of the drug thalidomide had kept it on the market after evidence of disastrous side-effects had appeared; it courageously defended and won a libel suit brought by the manufacturers. Later, the *Sunday Times* told, of Israel's plutonium manufacturing plant at Dimona in the Negev Desert.

Sadly, Evans's innovation has now been replaced by opinionated commentary on matters in general. By contrast, Hodgkinson's espousal of Duesberg's opinions on AIDS superficially resembled investigative reporting; the articles were often written from faraway places and well, if selectively, supplied with quotations from people on the ground. He returned repeatedly to the same theme. Even now, it seems, Witherow is not entirely persuaded that this campaign was misguided. In a letter last Thursday to the *Independent* newspaper, he restates Hodgkinson's conviction that African AIDS is really tuberculosis, and that the use of amyl nitrate is linked with "the destruction of the immune system", Witherow has earned the denial of his assertions by a number of people who know better than he does. That is probably where the controversy will end.

What the British press should learn from this affair is that the institution of the Sunday newspaper carries inherent dangers. In simulating the appearance of news, sensational reporting may be mistaken for heroic journalism and bias for perceptive iconoclasm. It would be safer if the Sundays were managed, as in the United States, as extensions of their daily equivalents. Then, at least, those whose job is factual reporting would have a say in what their Sunday cousins write. □

Prions and public health

Britain should respond constructively to the German demand for a ban on imports of British beef.

NOBODY knows whether the agent responsible for bovine spongiform encephalopathy (BSE) can cause Creutzfeldt-Jacob disease (CJD), but there are enough people in Germany who believe that transmission from cattle to people is a possibility for the British government to be embarrassed. The Ministry of Health in Bonn is seeking a ban on the import of British beef to Germany.

The British government's opposition to the ban has been weakened by the revelation, last week, that the authorities have decided to extend present restrictions on the use of certain bovine offal as human food to that from calves younger than six months. The decision follows a feeding trial in which calves were given large doses of the infectious agent in the form of brain tissue from infected adult animals, and were found to have infectious agent in some tissues by means of a biological assay using mice. The chief economic consequence will be in the export trade in live calves for veal production elsewhere. But the psychological effect on the British beef industry is bound to be considerable.

Unfairly, perhaps, but not unnaturally. Nobody will be greatly surprised that even calves can be infected with BSE. If the infectious agent is a prion, a protein molecule, the chance of infection must be presumed to be positively related to the dose an animal receives. But the latest development does not in itself undermine the British strategy for controlling BSE, which since 1988 has banned the use of sheep offal in the manufacture of cattle feed. The strategy is based on the assumption that the British cattle herd was first infected by means of tissues from sheep themselves infected with scrapie. That strategy should be effective provided that there is neither maternal transmission *in utero* or by milk nor the infection of one animal in a herd by another.

British ministers last week claimed a sharp decrease in the number of cases of BSE among cattle less than five years old. But if there is no maternal transmission, the incidence of the disease among such young animals should now be literally zero. Allowing for the use of some contaminated feed after the ban was introduced, the incidence should tail off to zero very soon. That is the crucial evidence on which the future of the British beef industry will rest. Fortunately for British farmers, another year should clinch the question.

What, meanwhile, of the proposed German ban on imports of British beef? If it were not that both countries are members of the EU, Germany need have shown no restraint. But it would be ridiculous for Britain to look to the EU to take Germany to the European Court to declare the ban illegal. Much better that it should invite the collaboration of its fellow-members of the EU in the monitoring of the incidence of BSE in British cattle. Better still, why not acknowledge that BSE is unlikely to be the last hypothetical threat to public health by prions, and resolve that a directed research programme should be one of Europe's urgent needs? □

Europe plans convention on social impacts of biomedical technologies

Paris. The rights of the individual should take precedence over those of science and society, according to the draft of a proposed 'European Convention on Bioethics' released last week by the 32-member Council of Europe.

The proposed convention is being billed as the first international treaty specifically to address the potential risks of progress in biology and medicine to the rights and freedom of human individuals, as well as to the future of the species.

Achieving consensus on bioethical issues from 32 European countries is an impossible task, and the content of the convention is inevitably little more than a lowest common denominator of member states' wishes.

Nevertheless, supporters of the convention argue that it provides an umbrella of broad fundamental principles that member states are likely to agree to — probably next year — and then tailor to their national legislation, for example by adding greater detail or stricter terms.

The backbone of the convention is a statement that "safeguarding the fundamental rights and freedoms of individuals, and in particular their integrity, dignity and identity" is its main goal.

The safeguards it proposes rely on the principle of free and informed consent to treatments and experiments, protection against unlawful interference with the human body, a ban on using the body — or its

parts — for "financial gain", and restrictions on predictive genetic testing.

The convention also formally introduces the notion that society now needs to set the limits to scientific research. Scientific liberty is needed, it says, but only where this remains compatible with the "protection of humans"; developments in biology and medicine must be used only for the "benefit of present and future generations".

Member states failed to agree on a definition of a "person" or a "human being", opting instead for the principle that "human dignity must be respected from the beginning of life". Similarly, the draft convention does not take a position on what should be done with surplus embryos, or under what conditions, if any, preimplantation diagnosis of embryos conceived *in vitro* should be permitted. Both issues dominated the recent debate over bioethics legislation in France (see *Nature* 369, 599; 1994).

A Council of Europe official says these issues will be tackled during the drafting of protocols designed to complete the convention. One such protocol, due to be completed by 1996, will address all aspects of

Proposed European Convention on Bioethics

Extracts from Preliminary Draft

Article 2: The interests and welfare of the human being shall prevail over the sole interest of society and science.

Article 3: Any intervention in the health field, including research, must be carried out in accordance with relevant professional obligations and standards ...

Article 11: The human body and its parts shall not, as such, give rise to financial gain ...

Article 15: 1. Where research on embryos *in vitro* is allowed by law, such research may only be permitted in the case of embryos which have not been developed for more than 14 days.

2. The creation of human embryos solely for research purposes is prohibited

the "human embryo". The official adds that it may be impossible to reach a common European position on such controversial issues.

Although the draft convention does not take a position as such on embryo research, it gets round this problem by stating that "when it is permitted by law (as in some European countries), research on embryos *in vitro* can only be authorized on embryos which have not developed beyond 14 days". The convention also bans the production of embryos solely for research purposes — this would be allowed in certain circumstances in the United States under new proposals being drawn up there by an advisory committee (see page 8).

The convention also bans predictive genetic testing except for medical or biomedical research purposes, and only then if results were kept confidential. Insurance companies would, for example, be prohibited from refusing insurance policies on the basis of results of a genetic test, or a candidate's refusal to take one. Employers would be allowed to demand a genetic test only where people were at risk from a disease that would make them unsuitable for particular working conditions.

Genetic modification of humans would only be permitted for "preventative, diagnostic or therapeutic purposes", except gene therapy to alter behavioural characteristics which "do not constitute a disease".

The convention bans modifications that would affect the germ line and be transmitted to subsequent generations. But it accepts that exceptions to this ban could be permitted in the future.

Declan Butler

Future brightens for space station

Washington. The dark clouds that had been hanging over the future of the international space station appear to have lifted following last week's decision by the US House of Representatives to approve next year's funding of \$2.1 billion for the project.

The vote, which passed by an overwhelming margin, follows a vigorous campaign by the Clinton administration to make Russia's proposed participation in the project a key foreign policy objective.

The decision, approved by 278 votes to 155, virtually ensures the space station's survival in Congress until next summer, and may well take the controversial project — on which \$11 billion has already been spent — beyond the point of no return.

The space station passed last year by just one vote. But once members saw it was heading for victory this year, they realized that voting against would merely antagonize the administration, and the vote

became a landslide.

But the underlying factors behind the space station's easy victory were the state of US relations with Russia and the mood of Congress on deficit reduction (see *Nature* 368, 279; 1994).

Russia appears more politically stable than widely expected when its participation in the space station was proposed last autumn. And the deficit-cutting Congress which killed off the Superconducting Super Collider last summer has returned to its more natural state of reciprocal favours and shameless 'pork-barrelling'.

The bill that funds NASA now moves on to the Senate, where Senator Barbara Mikulski (Democrat, Maryland) has promised to give NASA a hard time unless the administration offers her backing on other unrelated matters. But the medium-term prospects for the space station have seldom looked so bright.

Colin MacIlwain

Agreement urged on access to DNA databases

Paris. A French government committee set up to study the commercial use of DNA banks last week suggested that the scientists who created such a bank should enjoy privileged access for a period of three years.

After this, however, the committee said that the body that had paid for the bank (referred to as the 'sponsor') should have the right to open it to a third party. In a separate move, it has also called for a formal international agreement guaranteeing open access to all DNA sequence databases.

The committee was set up earlier this year following a public row over a proposal that would have given Millennium, a Massachusetts-based biotechnology company, exclusive access to a DNA bank held at the Centre d'Etudes du Polymorphisme Humain (CEPH) in Paris (see *Nature* 368, 175; 1994).

Philippe Froguel, the researcher who developed the bank from samples collected from more than 800 French families with a history of non-insulin-dependent diabetes, objected strongly to the proposal, accusing Daniel Cohen, the director of CEPH, of trying to "sell" the bank. Froguel, dismissed as a result of the dispute, argued that he should have control over access to the bank.

The committee's compromise acknowledges that the sponsor of a DNA bank should have 'legal responsibility' for it. But it also recommended that the researchers who built the bank should have a period in which to exploit the bank themselves.

According to the committee, the lack of clear contractual arrangements contributed directly to the CEPH dispute. It recommended that a new commission should be set up to approve all research on DNA sequences, assessing the scientific and ethical need for a bank, ensuring that the terms

of its eventual commercialization are explicitly agreed at the start of a project and arbitrating on any conflicts that arise.

In line with France's recently adopted bioethics bill (see *Nature* 369, 599; 1994), the committee said that donors to DNA banks should not be paid, nor be entitled to any royalties on the use of their DNA. But it also proposed that donors must agree to any use made of their DNA, in particular in commercially oriented applications.

The committee's recommendations on databases of DNA sequences that cannot be traced to individual donors are very different. Such databases, it says, represent "an international instrument of cooperation and

diffusion of knowledge", and must be shared from the outset.

The committee calls for a formal international agreement guaranteeing open access to all sequence databases, and defining the terms under which their costs and the construction are shared. Those supplying sequences to international databases would be given a 'copyright' acknowledging the originality of their contribution.

The report's recommendations on DNA banks, which are likely to be accepted as a reasonable compromise on all sides, will probably be introduced as decrees — expected to be applied retroactively — in the autumn.

Declan Butler

Généthon builds up 'genetic valley'

Paris. The Généthon laboratory near Paris and the biotechnology start-up company Genset last week launched a joint FF600 million (US\$11 million) programme to sequence and analyse all the regulatory regions of the human genome by the end of 1996.

Their aim is to identify transcription factors that activate genes, as well as their binding sites. Genset markets oligonucleotide-based products, and has developed transcription factor decoys — short DNA sequences that mimic regulatory sequences — which it hopes will compete with endogenous binding sites, preventing transcription factors from activating genes involved in disease.

Généthon was set up in 1990 jointly by the French Muscular Dystrophy Association (AFM) and the Centre d'Etudes du

Polymorphisme Humain (CEPH). Based at AFM's headquarters at Évry outside Paris, the laboratory has rapidly become one of Europe's leading gene mapping centres, having produced complete genetic and physical maps of the human genome.

Under their agreement, the two organizations will create a joint Très Grand Séquençage (TGS) laboratory at the Évry site. Twenty-five people will start work using Généthon's existing facilities, but they will have their own building next year. Jean Weissenbach, scientific director of Généthon, and Marc Vasseur, chief scientific officer at Genset, will lead the project.

Généthon's strategic alliance with Genset, in which it will take a FF10 million stake, is its first with a private company. The move is part of a plan to build a network of such alliances — known as Généthon-Industries — and create a 'genetic valley' at the Évry site. Several small French biotechnology companies have already expressed interest in moving to Évry.

AFM wants these companies to collaborate in the recently launched Généthon 2 programme, aimed at identifying genes, and the planned Généthon 3 programme, which would work out their role and function. AFM's overall goal is to speed up the development of genome therapeutics.

Généthon is already collaborating with Rhône-Poulenc Rorer (RPR) and is likely to become part of the company's planned gene therapy network.

At the launch of TGS, Bernard Barateau, the president of AFM, criticized the government for its "absence of a clear strategy [in research] on the human genome", and called on it to launch a big "Génome-Santé" programme. Only by coordinating public and private funds can France — and Europe — compete internationally, says Barateau. A parliamentary study group is said to support Barateau's strategy.

D. B.

Caution urged on genome manipulation

Paris. Pierre Chambon, the eminent French biologist and geneticist, last week called for a global moratorium lasting 50 years on all germ line manipulations of the human genome.

Writing in the newspaper *Le Monde*, Chambon, who was recently appointed to the chair of molecular genetics at the Collège de France in Paris, congratulated molecular biology on its progress over the past 40 years in unravelling the fundamental mechanisms of life.

But he expressed concern that transgenesis (genetic manipulation) could, by allowing directed genetic change, open the way to "an era of Lamarckian biological evolution". Chambon argued that although such applications are still "science fiction", the situation could change rapidly, for example if genes that delayed ageing

were to be discovered.

Biologists, warned Chambon, often show "unreflected optimism" about the nature of, and delay in, the application of their discoveries. But he also attacked the "emotive" arguments of pressure groups, calling for modern biology to be given a central place in the school curriculum.

A "period of reflection" is needed, said Chambon. He argued that "a 50-year global moratorium on manipulations of the human genome would seem to me a wise decision".

D. B.

IMAGE
UNAVAILABLE
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REASONS

Chambon: cautious.

Piquemal/Egnoy/Frank Spooner Pictures Ltd

'Fine words, but few deeds' in Clinton science statement

Washington. The Clinton administration is expected to publish a broad-ranging policy statement within the next few weeks, setting out the reasons for its support for basic science, and suggesting ways in which this support could be made more effective.

This follows last week's approval of the planned statement, being referred to as a white paper, at the first meeting of the National Science and Technology Council (NSTC), a new body created by President Bill Clinton last year to co-ordinate US science policy. The statement is expected to put forward various broad recommendations for government policy, emphasizing, for example, the need for government agencies to share the costs of new research laboratories and equipment.

But officials who have seen the document say that it avoids outlining research priorities, and contains few figures or directives for agencies (such as how cost-sharing could be required of them in practice). Nor does it deal with burning issues such as the congressional threat to cut funding for defence research in the universities (see *Nature* 369, 694; 1994).

As a result, the proposed statement is already causing scepticism in Congress. "There's a lot of philosophy in it, but no specifics," complains one staff director.

Nevertheless John Gibbons, the president's science adviser, said after the meeting on 29 June that unanimous endorsement of the statement was a "very positive start" for NSTC, and had "allowed the president to express his support for science and technology".

Preparation of the white paper was set in motion at the beginning of the year by the fundamental science subcommittee of NSTC, a cabinet-level body intended to oversee US science and technology programmes which this year will cost \$72 billion, one-seventh of the entire discretionary budget of the US government (*Nature* 366, 393; 1993).

A statement issued by the White House after last week's meeting said that Clinton told the cabinet ministers and agency heads who attended "to continue to work to reprioritize science and technology investments to match national goals".

The president added that other goals for the NSTC — most of whose work is done by its nine subcommittees, with the council itself expected to meet only rarely — were to forge better links with industry, to persuade the general public of the value of science, and to expand international science and technology co-operation.

According to some accounts, Vice-President Al Gore, who took over as chairman when Clinton left 30 minutes into the 80-

minute meeting, lived up to his reputation as a technophile by telling the group to share ideas about one of his pet projects, the "information superhighway".

Donna Shalala, the secretary of health, and Neal Lane, the director of the National Science Foundation, each took the opportunity to emphasize to Clinton the value of fundamental science. Shalala pointed out the economic as well as social benefits of biomedical research, while Lane referred to the need to build closer links between research and teaching in universities.

Others present at the closed meeting included Hazel O'Leary, the secretary of energy, Richard Riley, the secretary of education, Federico Peña, the secretary of transportation, deputy defence secretary John Deutch, Dan Goldin, administrator of the National Aeronautics and Space Administration, and Carol Browner, administrator of the Environmental Protection Agency.

Also in attendance were Leon Panetta, newly appointed White House chief of staff, Alice Rivlin, his successor at the Office of Management and Budget, James Woolsey, head of the Central Intelligence Agency, and Laura Tyson, the president's economics adviser. Harold Varmus, the director of National Institutes of Health, having fought successfully for a seat on the council, was on holiday, and was represented by his deputy, Ruth Kirschstein.

Apart from the white paper, which most people in the room had already approved, the meeting endorsed the need to enable and encourage more minorities and women to enter scientific careers, and for agencies and departments to work more closely together.

The science white paper was originally drafted for the White House by the head of physics at the Massachusetts Institute of Technology, Ernest Moniz. According to officials who have read it, the statement is said to be considerably less specific than a corresponding document on technology produced last year.

One congressional staff member says that the original draft has experienced "homogenization" at the request of different interested agencies so that "it ends up just pontificating, which is not what we need; we get enough of that already".

Defenders of the white paper say that it has fulfilled its main task, namely explaining why the government should continue to invest in fundamental science. But, as one official acknowledges: "People expecting it to propose a whole new way of doing business will perhaps be disappointed." There is, after all, no broad consensus that American science is broken — let alone on how to fix it if it is.

Colin Macilwain

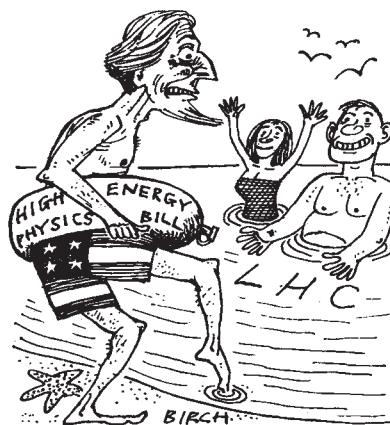
LHC's backers in Congress prepare to take on critics

Washington. Leading members of the House of Representatives' science, space and technology committee have put forward a bill backing the involvement of the United States in the construction of Europe's planned Large Hadron Collider (LHC).

The bill endorses budgets for high-energy physics at the level proposed in May by an advisory panel chaired by Sidney Drell of the Stanford Linear Accelerator Center (see *Nature* 369, 266; 1994). The panel said that physics needed an extra \$150 million over the three years from 1996 to allow the United States to fund its domestic programme, while providing initial support for the LHC.

The high-energy physics bill also directs Hazel O'Leary, the secretary of energy, to negotiate with the European Laboratory for Particle Physics (CERN) on US involvement in planning and construction of LHC.

But it also sets conditions on any resultant agreement, including a fixed ceiling on the US contribution and provision for US



withdrawal if benchmarks are not met.

Introducing the bill, committee chairman George Brown said that a successful experience in international collaboration at CERN was needed "to change the impression that the United States is not a reliable international partner" in big science projects.

The bill is also sponsored by science subcommittee chairman Rick Boucher (Democrat, Virginia) and by Sherwood Boehlert (Republican, New York), both of whom played a key role in halting the Superconducting Super Collider last summer.

The bill is by no means certain to pass the House. And even if it does, it would not guarantee US funding for LHC, as this is set annually in the Congressional budget. But its introduction indicates solid Congressional support for the LHC, ready to take on detractors who argue that, as there was no European support for SSC, there should be no US support for CERN.

C. M.

Lay-offs follow suspension of clinical trials of protein

London. Shares in the biotechnology start-up company Regeneron Pharmaceuticals Inc, have fallen dramatically in value following the company's announcement that it has halted Phase III clinical trials of the protein ciliary neurotrophic factor (CNTF).

The company abandoned the trials, which it had been hoping would lead to a potential treatment for motor neurone disease, after it had come to the conclusion that a modification of the trial suggested by interim results reported in March would not be sufficient to eliminate the problems encountered.

As a result, the company will reduce its staff by 25 per cent to about 200. Most of those to leave have been directly involved with the phase III trials of CNTF. The company will write-off approximately \$450,000 over the next financial quarter to cover the redundancies.

The announcement that the clinical trials had been suspended was followed by a 17 per cent fall in the value of the companies' shares. Since then, the shares have remained around this level, with only small fluctuations in their value.

The decision to abandon the nine-month-old trial was made after it began to reveal side-effects in some of the 720 patients taking part. These included weight loss, coughs, the suppression of appetite and flu-like symptoms.

CNTF is a cytosolic protein — one produced inside the cell — which has been shown to prevent the degeneration of neurones, both in animal models and in humans. Neurotrophic factors are seen as a potential treatment for amyotrophic lateral sclerosis (also known as motor neurone disease or MND), multiple sclerosis and other degenerative neurological diseases.

Regeneron had been testing CNTF for the treatment of MND, also known as Lou Gehrig's disease, a progressive degenerative disease of the motor neurones that causes gradual weakening and paralysis, initially in the arms and legs and eventually of the whole body. At present there is no treatment for MND, and the disease is fatal, usually within three to five years of diagnosis.

Michael Sendtner, of the department of neurochemistry at the Max-Planck-Institute for Psychiatry, who has worked extensively with CNTF in mice and rats, says he is not surprised by the trial results. "Our feeling is that they went too early to the clinic with the drugs," he says.

According to Sendtner, the potential side effects of the drug in clinical trials were discussed at a meeting in Birmingham in November 1992. The company "should have known" that there would be side effects, he says, suggesting that it might not have

carried out enough work on the pharmacokinetics of the drug in humans, rather than merely studying animal models, before proceeding to phase III trials.

But Len Schleifer, chairman and chief executive officer of Regeneron, rejects this charge. "In the context of an untreatable deadly disease, I feel that we had sufficient basis to move forward to phase III," he says. In retrospect, he adds, the major problem with the trial was that the side-effects "played havoc" with attempts to measure the effectiveness of the drug.

Rather than pursuing CNTF further, Regeneron now plans to concentrate on the company's second generation CNTF and other neurotrophic factors. Schleifer says that the kind of setback which the company has experienced is common in the development of ground-breaking drugs.

"The shame is that because one trial didn't work, people assume that neurotrophic factors are dead," he says. But he maintains that neurotrophic factors are still the most promising avenue for the treatment of degenerative neurological diseases.

At the same time, however, the company is also fighting a class action lawsuit brought against it in March — when the initial results of the trials were announced indicating that it was running into problems — claiming that the company made "false and misleading" statements about the CNTF study.

According to the suit, this was done in order to "create and prolong the illusion of Regeneron's success in the biopharmaceutical industry, to inflate the price of the common stock of the company and to conceal the adverse facts concerning the company's future business prospects".

Schleifer claims that the shareholders are "totally baseless in what they say", and dismisses as "ludicrous" the suggestion that the company knew that the clinical trials would not work. "We were totally blind until shortly before the announcement," says Schleifer, adding that he plans to contest the action "vigorously".

Maggie Verrall

UK research council splits peer review from policy inputs

London. Britain's new Engineering and Physical Sciences Research Council (EPSRC) has unveiled details of a radical reorganization of its system for allocating research grants which will, for the first time, distinguish between its procedures for assessing grant applications and those for establishing research priorities.

Under the former Science and Engineering Research Council (SERC), which was abolished on March 31 this year, scientific committees had been responsible both for judging the scientific content of all applications received in their field of competence, and for making recommendations to the council about future funding for the field.

The EPSRC, created out of the break-up of the SERC following last year's white paper on science, gave its approval last week to a new set of procedures which, it hopes, will streamline both the grant review and the policy making processes, but will also create a "Chinese wall" between the two activities (see *Nature* 396, 3; 1994).

All grant applications will, once they have passed an initial screening by three referees, be assessed by an *ad hoc* review panel whose members will be drawn from a 30-40 member 'college'.

Proposals will be ranked by this panel, in discussion with a programme manager from the EPSRC, who will also remind panels of the research councils strategic objectives. Once the ranking has been agreed, the funding of a particular application will depend on the amount of money which the council has made available for the field.

Inputs into discussions on future council policy, which were previously provided by the peer review committees, will now arrive by a separate route, namely through a "technical opportunities panel" responsible for identifying areas of scientific promise.

The so-called TOP panel will operate in parallel with a second 'users panel', and advice from the two panels will be used as the basis for the research council's funding applications from the government. □

OECD confirms drop in Japan's R&D spending

Paris. Figures released last week by the Organization for Economic Cooperation and Development in Paris have confirmed that, after ten years of rapid and continuous growth, funding for research and development (R&D) in Japan fell sharply for the first time in 1992.

The decline in Japan followed that which had appeared at the end of the 1980s in virtually all major Western economies. It was primarily the result of

reduced R&D expenditures by Japanese industry, which fell by almost three per cent between 1991 and 1992 (see *Nature* 366, 97; 1993).

Overall spending on R&D by Japan dropped from 2.87 per cent of its gross domestic product (GDP) in 1991 to 2.80 in 1992. In contrast, US spending, which had fallen by 3.5 per cent from 1990 to 2.67 per cent of GDP in 1991, increased to reach 2.68 per cent of GDP in 1992. □

Regional needs save environmental projects

Munich. A German laboratory that has played a prominent role in addressing the environmental problems of East Germany is to be allowed to continue working in the environmental field, despite the recommendations of a top-level scientific committee that such work be wound up.

The proposal to close down work at the GKSS research centre near Hamburg, one of 16 national research laboratories in Germany, was made in a report on environmental research put together by a scientific subcommittee of the Wissenschaftsrat, the country's main science advisory body.

But, following criticism of the subcommittee's conclusions by the local region, the Wissenschaftsrat has now agreed that the centre should merely lose one of its outstations in east Germany, the Magdeburg-based Institute for Water Research. It will be allowed to continue its work on monitoring and assessing environmental damage.

The GKSS was established in 1956 to carry out research and development on nuclear-powered ships. But, like many other nuclear research facilities, it had to find new goals in the 1970s, and shifted nearly half of its activities into environmental research. It has also built up programmes in materials research and separation techniques.

According to the Wissenschaftsrat's science committee, which has just completed a two-year survey of environmental research in Germany, the change in direction had not been successful. "The research was just very weak," says Gotthilf Hempel, who headed the committee. "There were too many groups doing different things that were not very innovative and there was virtually no collaborative work."

As well as involving scientists, the main committee of the Wissenschaftsrat is made up of politicians from the federal and state (*Länder*) planning departments. Accommodating political pressure is a fact of life for the Wissenschaftsrat, according to its former head, zoologist Gerhard Neuweiler. As a result, the recommendations of scientific subcommittees are endorsed only if they receive broad political approval as well.

Not surprisingly, the more drastic of the environmental science committee's recommendations were apparently modified in the light of pressure on the main committee. In particular, the state of Schleswig-Holstein, where GKSS is located, opposed major cuts in its national research centre.

As a result, a compromise was reached. It was agreed that the centre should be allowed to continue with environmental research if it reduced the number of research projects and focused on its acknowledged strengths in environmental monitoring.

In fact the GKSS has already been moving in that direction. At the end of 1992, Günter von Sengbusch, an industrial medical engineer, became the centre's director and slowly gained the confidence of committee members. On the basis of an analysis of the centre's activities by external consultants, whose conclusions broadly coincided with those of the Wissenschaftsrat, he began to cut the number of research projects and

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Pollution remains a problem in East Germany.

concentrate the centre's environmental activities on the management of coastal ecosystems in the Hamburg region.

The Wissenschaftsrat appears to have endorsed von Sengbusch's actions. But in a move that von Sengbusch describes as "regretful", its east German outpost, the Institute for Water Research, will transfer to the new national research centre for environmental research (UFZ) in Leipzig-Halle.

The institute had already been rescued once by the Wissenschaftsrat when it recommended two years ago as part of an evaluation of all east German research that the institute's two major research activities be saved. These concern the ecology of the badly polluted river Elbe, and the control of non-natural lakes on sites of abandoned coal mines.

The Wissenschaftsrat recommended that the institute either be established as an independent 'blue-list' institute — one for which the federal and regional governments share the costs equally — or merged with the newly established UFZ.

At the time, the GKSS, which is located

at the Elbe estuary, successfully argued for a third alternative — that the two centres in west and east Germany that studied the river Elbe should be united.

Reinhold Leitterstorf, the head of the research ministry's environment department, now argues that the ministry "made a mistake" in ignoring the Wissenschaftsrat's advice and accepting the GKSS's plan. The Institute for Water Research will now be transferred from the beginning of January.

But the institute's director, Walter Geller, argues that the merger with the GKSS was appropriate for the time, since it saved the relatively poor *Land* of Sachsen-Anhalt a considerable amount of money (the federal government picks up 90 per cent of the bill for national research centres), the UFZ was still establishing itself, and the GKSS provided valuable technical support for water analysis, for which the institute was ill-equipped.

Geller says he is now equally happy with the institute's transfer to the UFZ, whose major task is to analyse and reverse the extensive environmental damage in eastern Germany. The move will allow the institute to build links with other UFZ programmes, such as those on the environmental impact of mines producing brown coal.

The GKSS was not the only target for criticism in the Wissenschaftsrat's report. Environmental research overall, which is generously funded, was criticized for being too fragmented, lacking the collaboration between research teams needed to raise the standard of their work to international level.

Such lack of collaboration, it said, was particularly apparent between university-based research groups. Neuweiler argues that it is one reason why, despite some pockets of excellence, "Germany is not as internationally renowned in ecology as it should be".

The new report also suggests that a greater proportion of the funds now allocated for environmental research at the national research centres should be made available on a competitive basis to scientific groups in research institutes and universities.

Alison Abbott

Italian to head bioinformatics institute

Munich. The director of the European Bioinformatics Institute (EBI) in Cambridge, UK, the new outstation of the European Molecular Biology Laboratory (EMBL), is to be Paolo Zanella, a former head of computing services at the European Laboratory for Particle Physics (CERN) in Geneva.

The 60-year-old Zanella is currently research director of the Centro di Ricerca Sviluppo e Studi Superiori, a high per-

formance computer centre which undertakes research and development for industry and research centres in Italy. Zanella helped to set up the centre, which is located in Sardinia, on leaving CERN in 1989 and still teaches at the University of Geneva.

The EBI will house and extend the European mammalian gene database, currently at EMBL's main laboratory in Heidelberg.

Alison Abbott

US panel firms up views on embryo research

Washington. An advisory panel to the director of the US National Institutes of Health (NIH) is likely to recommend approval for experiments on human embryos up to the fourteenth day after fertilization.

It is also expected to back the approval under certain circumstances of the deliberate creation of embryos for research purposes, but to defer a recommendation on the use of oocytes from fetuses until the issues raised have been studied in more detail.

At its fifth — and probably final — meeting last month, the NIH's Human Embryo Research Panel fleshed out its proposed new guidelines governing the use of human embryos in federally funded research.

The use of federal funds for such research became possible only last June, when Congress cancelled a requirement for all federally funded *in vitro* fertilization (IVF) research to be reviewed by an ethics advisory board. Because of links between the research and the abortion debate, the board ceased to function during the Reagan and Bush administrations, leading to a *de facto* ban on the use of federal funds for such research.

The panel is due to deliver its report to the NIH director, Harold Varmus in September for consideration by his advisory committee later this year. It was asked to identify areas of research considered acceptable for funding, those that are more troublesome from an ethical and moral standpoint and therefore warrant additional review, and those considered wholly unacceptable for federal support.

On the basis of a series of straw polls at the meeting, and with its public deliberations now over, the panel seems likely to recommend that all proposals falling within the 'acceptable' category be subject to some form of national review by an *ad hoc* body. This intentionally vague recommendation leaves room for Varmus to decide whether such a body would have primarily a monitoring or a 'gatekeeping' function.

The panel is also likely to recommend approval of the creation of embryos expressly for research purposes — rather than limiting research to 'spare' embryos from

IVF programmes — either for fertilization studies, for use as controls, or where there is a compelling scientific need. But it voted to prohibit the payment of donors, and the use of gametes and/or embryos without the donors' consent.

The panel voted to allow the development of embryonic stem cell lines by a small margin, after hearing of the scientific potential of this work.

In line with countries such as Australia, Canada, Sweden and the United Kingdom, the panel recommended a 14-day limit on human embryo research after fertilization. This is the point at which the primitive streak can be identified *in vivo*, and some consider an individual's 'identity' is established.

The panel seems likely to side-step the controversial issue of whether to allow fetal ovaries to be used as a source of oocytes for the creation of research embryos, pending a more extensive review of the ethical issues.

It is also likely to propose further review on the cloning of human preimplantation embryos by either separating blastomeres or splitting blastocysts (twinning), and on the use of embryos for research after the appearance of the primitive streak, but before closure of the neural tube.

Areas expected to be declared off-limits include the creation of chimaeras, cross-species fertilization (except to test the viability of human sperm), the implantation of parthenogenetically activated human oocytes and the use of preimplantation genetic diagnosis for sex selection (except to identify sex-linked diseases).

The panel's activities have not gone unnoticed by 'pro-life' groups, or by their congressional supporters. The International Foundation for Genetic Research/Michael Fund of Pittsburgh, Pennsylvania, for example, has filed a lawsuit against Donna Shalala, the health secretary, Varmus and all 19 panel members, attempting to halt the panel's deliberations and shift the debate from NIH to Congress.

And in a letter sent in mid-June to Varmus, co-signed by 34 mainly Republican mem-

bers of the House of Representatives, Robert Dornan (Republican, California), outlined several areas of research that he said "Congress has never discussed, and which pose grave ethical problems of their own". These, he said, include the use of oocytes from the ovaries of aborted fetuses, and the use of preimplantation diagnosis for the purposes of sex selection and genetic diagnosis.

Dornan also criticized the make-up of the panel, claiming that it was biased in favour of those with an interest in the research continuing. In reply, Varmus wrote that panel members were not picked as "proponents of specific points of view about human embryo research, and they were not asked in advance for their positions on the acceptability of this research."

Meanwhile NIH has received more than 17,000 written comments, many raising the abortion issue. Most of the letters are opposed to any form of human embryo research. But Anne Thomas, a spokeswoman for NIH, says many of the comments are not pertinent to the work of this panel.

Diane Gershon

Weapons scientists back cuts in new nuclear systems

Washington. Almost two-thirds of scientists working in weapons laboratories supported by the US Department of Energy would support a reduction in federal support for the development and testing of new nuclear weapons, according to a survey carried out on behalf of Sandia National Laboratories.

This is only slightly less than the 68 per cent of the general public which the survey, carried out by researchers at the University of New Mexico's Institute for Public Policy and the Georgia Institute of Technology, found also supported such a cutback.

Not surprisingly, however, it contrasted with the 96 per cent of members of the Union of Concerned Scientists (UCS) polled who shared this view. Similarly, while 22 per cent of the laboratories' scientists (and 31 per cent of the public) favoured cuts in existing weapons, this view was held by 75 per cent of UCS members.

Seven out of ten members of the first two groups agreed that more research is justified on increasing the safety of existing weapons, indicating general support for the type of activities that the national laboratories are keen to remain involved with.

Furthermore, a clear majority in each group (including 60 per cent of the UCS respondents) backed the idea that more money should be spent on providing training for those required to handle weapons in the nuclear arsenal. □

Rees appointed Astronomer Royal

London. **The Royal Society announced last week that Sir Martin Rees (right) will succeed Professor Arnold Wolfendale as Astronomer Royal, a largely honorary post created in the seventeenth century and combined up to 1971 with the directorship of the Royal Greenwich Observatory. Rees is Royal Society Professor at the Institute of Astronomy in Cambridge. He takes up the post in January, and is also next year's president of the British Association for the Advancement of Science. The Royal Society has welcomed Rees's appointment, claiming that he will be "well able to act as a spokesman for the astronomical community" in Britain.**

Intellectual property dispute hits US-India science accord

New Delhi. The Indian government appears to be dragging its feet over accepting an offer from the United States first made seven months ago to extend collaboration in science and technology between the two countries after the current phase of cooperation comes to an end in three years' time.

The main point still at issue is apparently a US demand that India revise its laws on intellectual property to cover all joint research projects that would be funded under the new agreement.

The new agreement was put forward by Elinor Constable, US assistant secretary of state for international environmental and scientific affairs, during a visit to New Delhi last November. She claimed that it would lead to an expansion in Indo-US science cooperation into the next century, and said that she looked forward to its "negotiation and approval".

Some 50 joint research projects are currently financed from the US-India fund, established in 1987 in rupees with a value equivalent to US\$110 million, and held by the American Embassy in Delhi. The fund will be exhausted by 1997, and the latest US proposal is intended to maintain cooperation on an even larger scale than previously through joint funding of projects by the two countries.

According to Indian officials, the agreement has still not been signed because the two sides have so far failed to reach agreement on the issue of intellectual property rights (IPR). The US proposal contains a proviso stating that, unless the Indian patent law is changed, its scientists should not be allowed to embark on joint projects with Indians if the results might become commercially exploitable.

At present the Indian law of intellectual property gives protection to processes and not products. As a signatory to the general agreement on tariffs and trade (GATT), India is obliged to amend its law. Officials say the transition to the new regime will take five years. But the United States wants India to settle the IPR issue bilaterally as a pre-condition for renewed cooperation in science and technology.

The earlier Science and Technology Initiative, launched in 1983, was not renewed after 1991 because of differences over IPR, while the much publicized vaccine action programme introduced in 1988 was allowed to lapse for the same reason.

The delays in signing the new agreement have led many observers to conclude that the dynamics of cooperation between the two countries, which have changed frequently over the past 35 years, are once again on the wane. **K. S. Jayaraman**

Earthquake 'forecasters' face their critics in Japan

Tokyo. A leading critic of Japan's earthquake prediction programme claimed last week that such prediction is not scientifically possible, that there are no reliable precursors of earthquakes, and that scientists should not mislead the Japanese public into believing that they can be predicted.

Robert Geller of Tokyo University was given, together with other critics of the programme, a rare public opportunity to express his views at a symposium in Tokyo sponsored by the Japan Science Council and the Seismological Society of Japan.

The prediction programme has consumed hundreds of millions of dollars over the past three decades without predicting any earthquakes. But even with the criticism expressed at the meeting, it seems unlikely that there will be any major changes.

Japan is the only country in the world to maintain a major research programme in earthquake prediction. The Japanese government has set up an elaborate network of seismic instruments in the populous Tokai region southwest of Tokyo, in the belief that it will be possible to predict the next major earthquake in that region and issue a warning to the public.

The programme began in the early 1960s, and is now entering its seventh five-year phase. Despite severe criticisms by external reviewers (see *Nature*, 364, 370; 1993), it received a significant 30 per cent boost of its budget this fiscal year, to ¥10.6 billion (US\$109 million).

The symposium was one of a series on earthquake prediction held about every four years. It was the first to include both speakers and organizers from outside the programme. Furthermore, supporters of earthquake prediction took a back seat and confined their interventions to brief five-minute comments on presentations by their critics.

In reply to Geller, Harumi Aoki of Nagoya University, who heads the Coordination Committee for Earthquake Prediction Research in Universities, argued that Geller's definition of prediction, requiring the time, location and magnitude of an earthquake to be narrowly defined, is too restrictive.

Aoki said that other forms of prediction, such as long-term predictions and those about the likely size and position of earthquakes without specifying the precise time, are also useful to the public.

Hiroshi Wakita of Tokyo University, another leader of the prediction programme, vigorously defended his research on radon. Geller produced records of radon measurements by Wakita, which seemed to indicate no reliable pattern between the occurrence

of radon anomalies and of earthquakes.

But Wakita said that each anomaly must be looked at individually, taking into account background noise caused by weather and non-seismic crustal deformation, in order to interpret his data correctly. He also pointed out that he had published a paper in *Science* that had been seen by three referees.

While other critics were milder in their calls for reform, Geller said that the pro-

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Last year's earthquake came without warning.

gramme should be replaced with a basic research programme in seismology that is not given the impossible goal of predicting earthquakes.

But even many of his supporters feel this view is too extreme, and that a wide-ranging reform of the existing programme would be sufficient. They argue that cancellation could result in funds being diverted to Japan's disaster prevention programme, which industry is keen to see expanded, and support would thus be lost for research.

In practice, cancellation seems unlikely for at least five years. Reformers had drawn hope from a comment from Yukiko Hirakawa, the Ministry of Education, Science and Culture's planning director of global environment research. She recently took administrative responsibility for the prediction programme, and was quoted in the 17 June issue of *Science* as saying "I'm embarrassed to admit this, but until I took over this position I thought earthquake prediction was much more advanced".

But after what she describes as a "deep study of the programme", Hirakawa says that she has now changed her opinion. "Although there are many problems to be solved to achieve a practical method of earthquake prediction, society's expectations are so high that the programme is most unlikely to end", she says.

Hirakawa claims that Aoki's rebuttal of Geller's criticisms was "very convincing", adding that the government has "absolutely no intention" of revising the programme from the start of the seventh five-year phase.

David Swinbanks

Hamsters and interferon

SIR — It is a pity that in writing about Hayashibara's successful lawsuit against Roche (*Nature* 368, 486; 1994) you highlighted the use of hamsters in our production of interferon in a way that may cause misunderstanding.

For the manufacture of interferon, there is, besides the Hayashibara method, an *in vitro* culturing (tank culture) method in which a huge amount of fetal calf serum is required. In 1992, about 180,000 fetal calves were killed for the manufacture of interferon by this method in Japan alone. About half a million calves are slaughtered worldwide to make bioactive substances in this way. Many experimental animals are used in medical research and in developing drugs and cosmetics. It is unfair to single out Hayashibara for criticism. Moreover, the cartoon that accompanies your news story shows little understanding of the respect and remorse shown towards animals killed for the benefit of humans here in the East.

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SIR — I was a member of the first Biogen team to produce recombinant human interferons in *E. coli* between 1980 and 1984, and would like to comment on interferon production using lymphoblastoid cells grown in hamsters.

(1) When we started large-scale production, our expression levels were of the order of $1-3 \times 10^8$ interferon units per litre of bacterial culture fluid. Current yields are at least a factor of 100 higher than this. According to your recent news item, one hamster can produce $2-4 \times 10^7$ units. Even with the low titres we had in 1981, one litre of culture fluid produced more interferon than five of the Hayashibara hamsters. As we were running a 30,000 fermentor on a 24 hour "turn", in one day we could make more human interferon than 150,000 baby hamsters would produce in 3-4 weeks of painful tumour growth.

(2) Purified recombinant interferons are as active in humans at those isolated from tissues.

(3) Proteins isolated from human cancer cell culture have a much higher probability of contamination with mammalian cell components than those from bacteria.

(4) The process is out of date. Similar techniques for the production of monoclonal antibodies in rodents have been superseded by efficient methods to produce antibodies using *in vitro* hybridoma cell culture or bacteria.

This process would not be allowed in Switzerland or Germany as there are

alternatives to the production method¹. Even if we completely ignore the suffering to the animals and the human workers who must care for them, the Hayashibara process as described would never suffice to produce the interferon used today for treating human patients.

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1. Reinhardt, C. A. (ed.) *Alternatives to Animal Testing. New ways in the biomedical sciences, trends and progress* (VCH Publishers, Weinheim, Germany, 1994).

Charity work

SIR — Your leading article "Charitable research"¹ was not entirely accurate in its description of two underlying events.

First, the Charity Commission's proposals arising from an inquiry precipitated by a pressure group's complaint against two cancer research charities did not depend on the quality or otherwise of the published account of any particular piece of research that the charities had funded. The commission made no judgements of research quality, nor did it have the expertise to do so.

Second, your statement that the published report^{2,3} of a study of complementary medicine "is now acknowledged to have been misleading" suffers from the same deficiency as much of the media comment on this topic: it conflicts with the facts as recorded in the research literature. The report² may well have been misrepresented or misunderstood, but that does not make it misleading. It described a study of survival in breast cancer patients receiving complementary therapy compared with those who did not. The investigators from the Institute of Cancer Research had sought a randomized trial, but the Bristol Cancer Help Centre (which offers complementary therapy and was collaborating in the study) had declined on ethical grounds. The study design was therefore inherently imperfect. It was nevertheless approved for support by the peer-review mechanisms of the two funding charities. The report² commented on the drawbacks of an observational trial and the difficulties of matching patients. It was observed that survival rates (absolute or metastasis-free) of Bristol patients differed from the controls. Possible interpretations for this finding were discussed. The experimental design did not allow a definitive conclusion about causes, the report did not draw one, but subsequent media comment did.

The investigators responded³ to subsequent debate⁴ by reanalysing their data.

The difference between the Bristol patients and controls was lessened but remained statistically significant. There was still no evidence for any survival benefit of complementary therapy. The original² qualitative finding was thus confirmed³.

The Charity Commission has highlighted the responsibility of research charities for the quality of the research that they fund, and has proposed guidelines. There are two further sets of guidelines that the commission might usefully consider. One would cover the publicity launched by medical research charities for particular outcomes of their research. The other would apply to the claims made by alternative medicine charities when offering their therapies to the public.

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1. *Nature* 369, 428 (1994).
2. Begenal, F. S., et al. *Lancet* II, 606-610 (1990).
3. Chilvers, C. E. D. et al. *Lancet* II, 1185-1186 (1990).
4. Hayes, R. J., Smith P. G. & Carpenter, L. *Lancet* II, 1185 (1990).

Is Britain immune?

SIR — Your discussion of questionable research and scientific practices and analysis of the possible means of redress (*Nature* 369, 261-262; 1994) is written as though scientific misconduct were limited to the activities of US scientists. Why is discussion limited to foreigners, particularly Americans? The United States is to be congratulated on discussing and attempting to deal with this phenomenon through such bodies as the Office of Research Integrity (ORI). This machinery, for all its faults, does at least represent an acknowledgement that a problem exists and tries to deal with it.

No such machinery, formal or informal, exists in Britain. Yet in more than 30 years of clinical research in the field of Alzheimer's disease I have come across examples of each of the different categories of scientific misconduct you mention. Because of greater financial pressures on British researchers, some types of misconduct may be more frequent than others. For example a referee or member of a grant committee of one organization may be instrumental in delaying or refusing an application, only to submit a similar one elsewhere, or a referee may either delay acceptance of or turn down an application while largely replicating the work it reports and attempting to publish the results first.

Is it not time that the subject was more widely discussed as representing as much of a problem in the United Kingdom as it is elsewhere?

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A new look at male contraception

Carl Djerassi and S. P. Leibo

As a first step towards a new form of male contraception — sperm cryopreservation, vasectomy and eventual artificial insemination — the military services should begin a large-scale sperm cryopreservation programme.

THE prognosis for a new male contraceptive by the year 2000 is nil, because the development, testing and regulatory approval of a truly novel, systemic male contraceptive requires 15–20 years¹. Given the absence of serious research and development in male contraception by major pharmaceutical companies — and only they can bring such a product to the general public — the expectation for a 'male Pill' even after 2010 is dismal. Does this mean that condoms and *coitus interruptus* remain the only feasible options for 'reversible' male contraception?

Alternatives

One alternative is surgical sterilization, now among the most widely practised methods of birth control in the world, with more than 40 per cent of Chinese, 30 per cent of US and 23 per cent of UK couples having one sterilized partner². Consider the male sterilized partner: most men who have chosen this form of contraception (more than 500,000 annually in the United States) do so only after the birth of a child. Yet, because of divorce, many vasectomized men remarry while still young enough to desire a second family, creating demand for surgical reversal of sterilization³. Nevertheless, given its cost, the dearth of surgeons qualified to perform such microsurgery and the inevitable decline of fertility with time after vasectomy, surgical sterilization must for practical purposes be considered irreversible.

Why do two to three times more women than men choose sterilization, despite the fact that vasectomy, performed under local rather than general anaesthesia, is easier and less expensive than tubal ligation? Not only do males desire to keep their reproductive options open for much longer than is operatively and culturally feasible for women, but many also fear the real or imagined sequelae of vasectomy⁴, although, in general, vasectomy has been considered "the simplest and safest" form of sterilization by an advisory panel of the World Health Organization⁵. Although two large cohort studies concluded that vasectomy may increase the risk of prostate cancer⁶, a special review panel convened by the US National Institutes of Health reiterated that it must still be considered "safe and highly effective"⁷.

If successful reversal could be 'guaranteed', young men who have never fathered children might become candidates for vasectomy, transforming it into a

new method of reversible male contraception. Attempts to make vasectomy reversible by inserting valves or easily removable plugs into the vas deferens have proved to be unsuccessful¹. But there is another option, previously considered¹ but rejected for practical difficulties: collection and cryopreservation of semen before vasectomy. Recent techniques and data make this combination the only new form of reliable, male contraception potentially introducible within the next decade. As vasectomy and sperm cryopreservation are established clinical procedures, no new medicine, science or law would be required for young men to begin practising this form of contraception. Steps for persuasive demonstration of the method's efficacy are outlined below.

The key lies in the use of artificial insemination (AI) to produce pregnancy. Human fecundability (percentage of women becoming pregnant per menstrual cycle during which intercourse occurs) varies from 18 to 28 per cent⁸. A similar percentage is infertile (no pregnancy after one year of unprotected intercourse)⁹, prompting a burgeoning clinical practice and research in human reproductive biology, in contrast to the precipitous drop in research into contraception. For more than 25 years, male infertility has been primarily treated by the use of AI with fresh sperm, resulting in cumulative pregnancy rates of around 75 per cent¹⁰.

Because the optimum time for insemination can now be identified fairly accurately by home urine tests for detecting the rise in luteinizing hormone, the use of AI to treat infertility is increasing. A US survey¹¹ found that some 172,000 American women annually underwent AI, spending \$164 million to obtain the services of 11,000 physicians. Most women who undergo AI are undoubtedly seeking treatment for infertility, but the actual procedure (intrauterine, intracervical or intravaginal) is simple and takes minutes to perform¹⁰, often by nurse-practitioners or paraprofessionals. The most common method, especially for cryopreserved sperm, is to inject washed specimens directly into the cervical canal. In the United States, the cost ranges from \$250 for a single treatment to \$1,500 for a 'package deal' of five cycles of inseminations, including hormone measurements and sperm washing.

On a practical level, inseminations can be timed and used most efficiently with

sperm cryopreserved in liquid nitrogen at -196°C . Although pregnancy rates with frozen semen are lower than with fresh, the overwhelming logistic advantage of cryopreserved sperm is its at-call availability. Semen can be 'banked' for extended periods in \$1,000 liquid-nitrogen refrigerators (1,500-sample capacity) that can maintain their temperature of -196°C for almost a year without any electricity.

What has now changed on the male side of the reproductive equation to make the outlook for practical long-term storage of human semen so favourable?

In recent years, tens of thousands of pregnancies have been produced around the world with frozen-thawed sperm, 17,000 in France alone¹². (About 46 million head of cattle, as well as thousands of females of other domestic species, are annually inseminated with frozen semen¹³, attesting to the efficacy of the techniques of sperm cryopreservation.)

A 'normal' man's ejaculate of 2–5 ml may contain $20\text{--}60 \times 10^6$ sperm per ml, which is usually 'extended' or diluted with a cryoprotectant to yield several samples. If 20 million sperm were frozen, and only 1 per cent survived, the 200,000 survivors of a single sample might result in pregnancy, because even this low sperm concentration is sufficient for fertilization¹⁴. Intrauterine insemination, or induced ovulation by hormonal treatment, can significantly increase the likelihood of conception¹⁵. And with the newest methods of assisted reproduction, the injection of a single spermatozoon directly into the cytoplasm of a human oocyte (intracytoplasmic sperm injection, or ICSI) has resulted in high rates of fertilization and pregnancy¹⁶.

Viability

If young men are to agree to vasectomy, the 'shelf life' of cryopreserved sperm at very low temperatures becomes relevant. There is considerable evidence that sperm viability does not decline at -196°C (absence of thermally driven reactions) and that its fertilizing capability should persist for centuries. Women inseminated with sperm stored for more than 10 years have given birth to normal children¹⁷. Animal studies provide more extensive data. For instance, the lambing rate after AI of ewes with sperm cryopreserved for 5 years was the same as that with sperm frozen for 2 weeks¹⁸. Recently, bovine sperm frozen 37 years ago has been used to fertilize

oocytes, which developed normally¹⁹. More rigorous evidence of functional survival after extended storage in liquid nitrogen comes from experiments with cryopreserved mouse embryos. The functional survival of embryos stored for more than 1 year was almost identical to that of embryos stored for 13 years, and live mice have been produced from embryos stored in liquid nitrogen for this long²⁰.

Will cryopreserved sperm undergo genetic alteration in the frozen state? Use of frozen sperm by the entire international industry of cattle breeding rests on the assumption that cryopreservation does not cause genetic alterations, let alone damage to sperm. Hundreds of thousands of cattle bred by AI with frozen semen for specific phenotypic traits exhibit those traits. Cell death and chromosomal damage of tissue-culture cells subjected to various doses of X-irradiation in the frozen state at -196°C was about four times lower than at room temperature, leading to the estimate that cells stored in liquid nitrogen would require 32,000 years to accumulate damage equivalent to that caused by an acute dose of X-rays at 22°C (ref. 21). Direct cytogenetic analyses of human sperm found that cryopreservation did not cause any increase in chromosomal anomalies²². Collectively, these data indicate that cryopreservation is highly unlikely to cause any genetic alterations.

The advent of single-cell polymerase-chain-reaction methods²³ will make it possible to type and encode semen samples at the time of freezing and thawing, thus eliminating problems of mistaken identity¹. In addition, storage devices are being developed in the form of magnetic tape, carrying either bar or magnetic codes, made from plastic laminates (such as those used to package dry cereal) that are flexible in liquid nitrogen. Attaching cryopreserved semen samples to these films will enable many samples to be stored in the frozen state, yet to be retrieved as quickly as magnetic tapes are scanned by computer²⁴.

Vasectomy coupled with semen storage

will turn into 'reversible birth control' only if fertility of the stored sperm can be assured. Men who have already fathered a child present little difficulty, but what about those whose fertility is unknown? If their concerns can be addressed, then the pool of candidates may well include many post-pubescent males. So far, there is no way to guarantee fertility to all couples, but many procedures have been devised for predicting the fertility of semen specimens with an overall accuracy of more than 80 per cent^{25,26}. Such tests and the normal pregnancies resulting from single sperm injection¹⁶ show that sperm cryopreservation, coupled with the latest techniques of assisted human reproduction, should substantially increase the likelihood of even a male with very few sperm becoming a father — an impossible prospect by conventional sexual intercourse.

Clearly, this extension of vasectomy to a wide constituency will work only in relatively affluent countries where AI can be practised efficiently, and it is there that feasibility must first be established. It is in this context that we make the following proposal to start serious debate and possible implementation.

Until now, human birth control has been achieved by interfering at some point of the reproductive cycle of a fertile person. In the future — through vaccines and, probably much sooner, through vasectomy coupled with sperm cryopreservation — the 'normal' reproductive state of an adult may be *infertility*, a subsequent deliberate step being needed for fertilization. As mentioned, the scientific basis for accomplishing this in males is now with us. What is missing is confidence by a much wider circle of the medical community than reproductive specialists: by policymakers (including ethicists and legal experts), entrepreneurs, health insurers and, most importantly, potential male users. Note the absence on this list of the pharmaceutical industry and government regulatory agencies, as neither is likely to be involved in implementation.

Because most men are fertile, most of

the potential users will also be fertile. Before vasectomy, they will have been examined to verify that they produce sperm suitable for cryopreservation. As outlined above, the success rate of AI (to be covered by health insurance) in normal couples is now high. Nevertheless, the question remains: "But how do you *really* know what will happen on a large scale with preserved sperm over 5, 10, 15 . . . years?" The only convincing answer is to take the first step, *requiring no vasectomies*, through the establishment of comprehensive facilities for storage over many years of a vast number of human semen samples whose donors can be followed up in terms of their subsequent reproductive experience. We propose a swords-into-ploughshares initiative for financial, operational and motivational reasons:

- The military services are the source of the largest number of young men with detailed medical records and the potential for follow-up. With little difficulty and relatively minor expenditure, tens of thousands of volunteers could collect their own semen to be cryopreserved by the military for years. This step alone would generate an invaluable resource for studies on male fertility and for eventual spin-offs of the Human Genome Project.

- At triennial intervals, the military or public health services, or both, would subject these sperm samples to laboratory analyses^{25,26}, providing statistically meaningful and invaluable data on the fecundity of the stored specimens.

- Quinquennially, questionnaires would be distributed to the sperm donors, enquiring whether they had successfully fathered any children, or, if not, whether they had attempted to do so. Responses would be matched with accumulated records to establish the predictive value of the sperm tests.

- Men whose occupations place them at risk from genetic damage to their sperm might view sperm cryopreservation as a form of genetic insurance. This is certainly relevant to the armed services, where procreation after death in military conflicts might be an option, subject to legal definition of sperm ownership.

Only time will tell whether men — and women — will buy the idea of this new form of contraception. The indispensable first step is to start the clock running on a large-scale, long-term sperm cryopreservation programme combined with follow-up protocols. Once the requisite confidence in the technical, operational and legal-ethical questions has been established, one can envisage decentralized, entrepreneurially funded and operated programmes (covered by health insurance) taking on some or all of the components of a new approach to male birth control: sperm cryopreservation, vasectomy and artificial insemination. □

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Does folding determine protein configuration?

An ingenious account of the protein-folding problem turns the usual formulation on its head, yielding the suggestion that foldability is an evolutionary necessity.

THE problem of protein folding is notoriously one of the most difficult in biology. The starting point is the specification of the amino acids along the protein chain. There may be some hundreds of them altogether. In principle, each residue can interact with all others, so that there is a multitude of possible interactions. And many of the configurations implied by these random interactions are energetically metastable in the sense that their free energy is less than that of all configurations obtained from them by making all possible small displacements. But the free energy may nevertheless be far above the global minimum, if only that could be found. So the essence of the protein-folding problem is the question of how, in real life, a protein molecule of specified amino-acid sequence ends up in a unique configuration which, among other things, determines its biological function.

That is merely a statement of the general problem. In practice, there are other difficulties. As yet, for example, there is no systematic way of including the interaction of water molecules in the specification of the problem, but every reason to suppose that water interactions are of the essence in the function of real-life protein molecules. A further and related difficulty is that there is little hope of tackling the folding problem except at the level of atoms, of which there will be several hundreds, perhaps thousands, in a real protein. Given that, it is remarkable that people have done as well as they have in the past few years in bringing computation to bear on the problem.

Molecular dynamics has done most to make the problem approachable. The first need is for a force-field, or a specification of the interaction energy as a function of distance between all possible pairs of interacting atoms. (The peculiar difficulty arising with hydrogen bonds is that the force will depend on at least two distances, those between the proton and the two heavier atoms it is supposed to hold together.) Ideally, modellers should also allow the transfer of charge from any part of the whole molecule to any other.

In the world that lies a little further from the ideal, it would be good to tackle the problem from first principles, by calculating the geometrical configuration of the whole molecule and the structure of its valence electrons simultaneously, but a glance at any issue of the *Journal of the American Chemical Society* will show that the theoretical chemists are still struggling with

compounds comparable with a single amino acid in complexity.

Given a usable force-field, the problem can be handed over to a machine and to one of the intricate suites of software developed for the purpose. Such a program inevitably finds metastable states as well as that whose energy is absolutely the smallest. There are built-in techniques for testing whether a particular state is that of the smallest absolute energy. One is to give the molecule as a whole an abnormally high temperature to see whether the metastable configuration then migrates to one with less energy. 'Annealing' is the vivid term for that.

But a computer simulation is rarely a solution of a real physical problem. And the fundamental difficulty with the simulation of protein configurations is that, so long as the chosen force-field is necessarily a crude approximation to the truth, no amount of precision in the computation can give a person confidence that the state with the lowest energy is really the global minimum, let alone that it approximates to that of the native protein with any accuracy.

Given all this complexity (in the strict sense), it is not surprising that many biologists declare impatiently that nature cannot possibly afford the luxury of allowing each polypeptide made by the transcription of an RNA molecule from a ribosome to go through the processes of molecular dynamics until it reaches the configuration in which it is biologically active. Indeed, there is not time for that, as the late Cyrus Leventhal pointed out in the 1960s. So may it be that polypeptides begin folding once they have begun to be assembled at the ribosome, so that the final state is conditioned by the first part of the protein molecule to be constructed? Alternatively, people argue, there may be a protein (call it 'templin') whose function is to be a template that determines the folding pattern of a whole class of related proteins.

E. I. Shakhnovich, a Russian at the Chemistry Department at Harvard, is now advocating a scheme for similarly finessing the difficulties of the molecular dynamics, and which, when tested, may have interesting implications for the evolution of protein molecules (*Phys. Rev. Lett.* **24**, 3907–3910; 1994). In essence, he turns the problem of protein folding inside out. First, he says, choose a configuration for a notional protein molecule with a predetermined length and then ring all the possible changes on the amino-acid sequence until the energy of the

structure is the smallest.

To make the job manageable, he has worked with a notional polypeptide of 80 amino acids and with an arbitrarily chosen configuration in which neighbouring amino acids are on neighbouring vertices of a cubic $4 \times 4 \times 5$ lattice (so that every vertex is occupied). The force-field is similarly skeletal; amino acids interact with each other only when they are on neighbouring lattice points, when their mutual energy is simply a number determined by the character of the pair concerned.

It is then possible to use conventional molecular dynamics (in this case, a simple Monte Carlo variation of the configuration is sufficient) to follow the folding of the energetically optimum polypeptide. The outcome is dramatic. The notional polypeptide hunts about for a stable conformation for 6 million time-steps in the Monte Carlo simulation, without encountering metastable states en route, until it finally clicks into the form of the notionally native protein — the tracing of a closed graph on the 80-vertex cubic lattice that Shakhnovich had thought of in the first place.

The importance of this result is not what it says about the particular (and unreal) configuration described, but that it suggests that, among all possible configurations for a protein of specified length and sequence, there may be one that allows for unambiguous and comparatively rapid folding into the native form. And once that state has been attained, thermal fluctuations do not seriously disturb the configuration: Shakhnovich estimates that his 80-residue polypeptide is folded properly 95 per cent of the time, and unfolded for 5 per cent of it. He also says that there are ample unpublished calculations to support these conclusions.

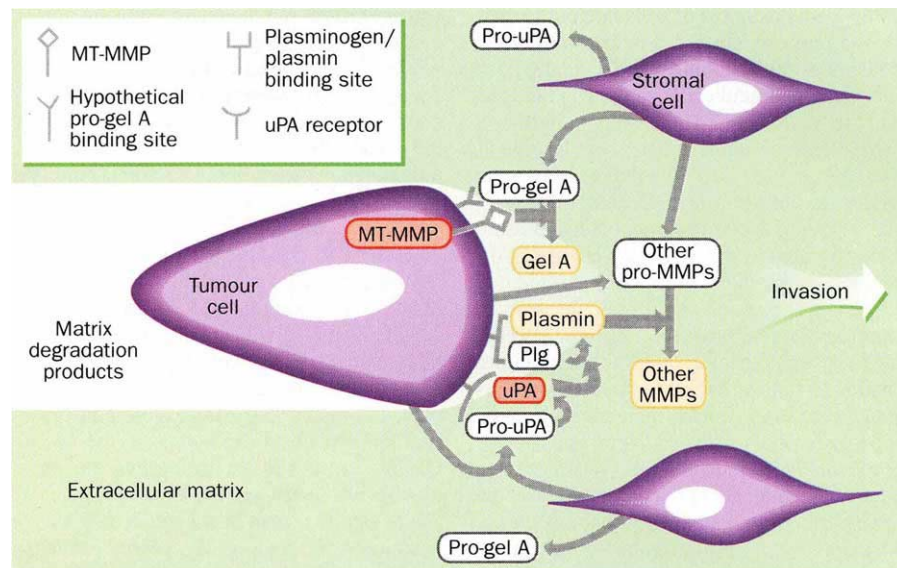
The novelty of the concept is that the protein configurations with which natural selection has endowed us may have been chosen not only for their biological activity but also for their capacity to fold unambiguously and in reasonably short order. (A protein that failed to fold would presumably be discarded quickly as a drag on fitness.) It will be particularly interesting to see whether this idea can be used to tell which amino acids at which sites in real proteins are likely to have been conserved in evolution. It will also be interesting to see the inevitable application of this line of argument to the secondary and tertiary structure of RNA molecules, where the same considerations arise. **John Maddox**

Membrane proteases in focus

Jean-Dominique Vassalli and Michael S. Pepper

THE extracellular matrix is an intricate mesh of proteinaceous fibres and carbohydrate macromolecules which must be crossed by cancer cells before they can invade tissues and metastasize. Proteolytic enzymes at the plasma membrane are likely accomplices in these processes, focusing degradative cascades close to the cell surface in order to clear a path for the

The catalytic activity of gelatinase A is tightly regulated. Like other MMPs, it is secreted as an inactive pro-enzyme, and tumour spread is correlated with increased levels of the activated enzyme⁸. But how is pro-gelatinase A activated? It is known that activation occurs in association with the plasma membrane⁹ and that pro-gelatinase A binds specifically to an as



Proteolysis on tumour cells. Invasion is facilitated by two classes of membrane-associated proteases which cooperate in degradation of the extracellular matrix. The matrix metalloproteinases (MMPs) include gelatinase A (Gel A), its precursor pro-gel A, and MT-MMP; enzymes of the plasminogen activator/plasmin system include urokinase-type plasminogen activator (uPA), plasmin, and their precursors pro-uPA and plasminogen (Plg).

invading cancer cell¹. On page 61 of this issue, Sato *et al.*² report the identification of a new membrane metalloproteinase with properties that equip it to be a ringleader in matrix destruction.

Cell migration is essential for development, inflammation and tissue repair, but it also allows malignant cells to exert their often lethal ability to invade tissues and metastasize. Localized degradation of the extracellular matrix is necessary for cells to migrate, and involves many proteolytic enzymes³. In view of the variety of substrates in the way, some of which (such as the fibrillar collagens) are extremely resistant to broad-spectrum proteases, this diversity is not surprising. Most of these enzymes belong to one of two families: the serine proteases, in particular the plasminogen activator/plasmin system⁴, and the matrix metalloproteinases (MMPs)^{5,6}. At least nine different MMPs have been identified: among these is gelatinase A (also known as 72K gelatinase or MMP-2), which also degrades collagen types IV and V, elastin and laminin⁵, and which is frequently overexpressed in stromal cells of malignant tumours⁷.

yet unidentified component of tumour-cell surfaces¹⁰, but so far the molecular features of these interactions have not been unravelled. The results of Sato *et al.*² go a long way towards clarifying the picture: using the power of primer degeneracy, they have identified an MMP that is an integral plasma membrane protein, the first membrane-type MMP (MT-MMP) to be discovered. Most important, MT-MMP can activate pro-gelatinase A and so might be the upstream activator in the gelatinase A proteolytic cascade. MT-MMP thus has two properties that will assist in cell migration: it is associated with the plasma membrane and so localizes matrix digestion to the vicinity of the cell surface, and it can activate the precursor of another extracellular protease and so set off an amplification of the destructive process. The relationship between the gelatinase A cell-surface binding site and MT-MMP remains to be explored.

Like pro-gelatinase A and its MT-MMP activator, the plasminogen activator/plasmin system is also implicated in invasion strategies. Expression of the urokinase-type plasminogen activator

(uPA) is often increased in malignant tumours, and like MMPs it fulfils the two criteria of plasma membrane localization and the capacity for catalytic amplification. uPA (or more precisely, its inactive precursor pro-uPA) is secreted as a soluble protein by tumour cells or by cells of the tumour stroma and binds to a specific glycosylphosphatidylinositol-anchored cell-surface receptor; this receptor is present on tumour cells¹¹ and focuses uPA activity close to the plasma membrane. Amplification starts with the activation by uPA of plasminogen, which is abundant in most extracellular fluids, cleaving it to plasmin. Plasmin is a protease of broad specificity which degrades, directly or indirectly through the activation of certain pro-MMPs, most if not all components of the extracellular matrix. Plasminogen and plasmin are also associated with plasma membranes, enabling a highly efficient proteolytic system to be assembled on the cell surface. Binding of uPA to its plasma membrane receptor enhances the cell's invasive potential¹², and preventing binding dramatically decreases metastasis in a transplanted tumour model¹³.

Another parallel between the MMP and plasminogen activator/plasmin systems concerns the theme of cooperation. Pro-gelatinase A can be activated by the new MT-MMP² and perhaps by uPA¹⁴, and other MMP precursors appear to require plasmin for their activation⁵. Stromal cells and malignant cells also cooperate to endow tumours with their destructive potential. Irrespective of which type of cell synthesizes the proteases or their precursors^{7,11}, which varies from tumour to tumour, increased extracellular proteolysis is a hallmark of aggressive malignancy.

The excitement caused by the identification of a new member of the MMP family with characteristics that set it apart from its congeners will spread outside the field of tumour biology. The cloning of MT-MMP from a placental cDNA library

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and the presence of its messenger RNA in normal lung demonstrate that it is not tumour-specific. What cell types express the enzyme? What other pathological processes are associated with alterations in MT-MMP expression? Extracellular proteolysis is a component of many inflammatory and degenerative diseases, and gelatinase A expression is widespread; for instance, this enzyme may be involved in amyloid-precursor-protein processing, and therefore possibly in Alzheimer's disease¹⁵. Some interesting questions relate to the catalytic activity of the enzyme as well: like other members of the family, MT-MMP has a 'pro'-domain that might confer latency. Does MT-MMP require activation? Besides pro-gelatinase A, are there other substrates for MT-MMP? Are there additional MT-MMPs for activation of other pro-MMPs? Might an MT-MMP be an activator of pro-uPA? Answers to these and similar questions should soon be forthcoming.

A more difficult issue relates to the relevance of these studies in the management of cancer. Because invasion fre-

quently occurs before any clinical manifestation, identification of the proteolytic enzymes involved is unlikely to help in preventing early metastasis. But mapping the proteolytic profile of human cancers may be valuable both as an index of prognosis and as a guide in the design of antiproteolytic strategies aimed at controlling progression of the disease. Extracellular proteolysis is also implicated in growth-factor activation³, which in turn may enhance proliferation of malignant cells, modify tumour stroma and trigger angiogenesis. Finally, proteolytically mediated changes of the extracellular matrix could affect the biology of its resident cells. For cancer and other diseases associated with altered extracellular proteolysis, details of how key enzymatic systems operate will be crucial. The identification of a membrane-type MMP is one important step in this direction. □

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COSMOLOGY

Intergalactic matters

Kenneth M. Lanzetta

It has been recognized since the early part of this century that interstellar space is filled with a tenuous mixture of gas and dust. But what about intergalactic space? Is there a diffuse intergalactic medium? Despite intensive observational efforts spanning more than thirty years, detection of the intergalactic medium has remained elusive. But a new Hubble Space Telescope (HST) observation, described by Jakobsen and colleagues on page 35 of this issue¹, provides what is very likely the first glimpse of the diffuse intergalactic medium. The observation is of an absorption feature imprinted on the far-ultraviolet spectrum of the high-redshift quasar Q0302-003. This breakthrough for cosmology provides the first direct evidence that the intergalactic medium is a highly ionized plasma of hydrogen and helium.

The possibility of using distant quasars to detect the diffuse intergalactic medium was first applied by Gunn and Peterson in 1965 (ref. 2). Their method made use of the Lyman- α resonance transition of neutral hydrogen, which occurs at a laboratory wavelength of 1,216 Å. They reasoned that in an expanding Universe a smoothly distributed sea of neutral hydrogen would absorb light from a background quasar of redshift z at all wavelengths between 1,216 Å and $(1+z) \times 1,216$ Å. By noting the absence of this distinctive absorption feature in the spectrum of the quasar 3C9,

Gunn and Peterson were able to limit the density of neutral hydrogen in the intergalactic medium to less than about 5×10^{-7} times the current 'critical' density needed to close the Universe. Subsequent observations improved on the sensitivity of the measurement and extended the method to include molecular hydrogen and neutral helium, but still no intergalactic medium was found. If there is an intergalactic medium, then it must be highly ionized.

A highly ionized intergalactic medium is certainly not implausible — ultraviolet radiation from quasars and young galaxies (together, perhaps, with mechanical energy released during the processes of galaxy formation) might sustain the ionization. But ionized hydrogen has no electrons, hence no electronic transitions, hence is not readily detectable. So how to detect all ionized intergalactic medium? The answer is by observing singly ionized helium. Singly ionized helium is similar to neutral hydrogen in that it has only one electron, although it has a nucleus of twice the electric charge and four times the mass of the hydrogen nucleus. That puts the 'Lyman- α ' resonance transition of singly ionized helium at a laboratory wavelength of 304 Å. Theoretical models of the intergalactic medium^{3,4} allow a wide range of ionization conditions, but many predict sufficient singly ionized helium to produce strong Gunn-Peterson absorption.

But the HST is sensitive to ultraviolet light only at wavelengths above 1,250 Å, so the helium 304 Å transition can be seen only at redshifts satisfying $(1+z) \times 304 \text{ Å} > 1,250 \text{ Å}$, or $z > 3.1$. At these redshifts, the problem is the 'Lyman continuum' ionization threshold of neutral hydrogen, which occurs at a laboratory wavelength of 912 Å. Ionization of neutral hydrogen produces a continuous absorption spectrum because recoil of the liberated electron can take up any energy of the incoming photon in excess of that required to ionize the atom. So, a foreground source of neutral hydrogen of redshift z — say the outskirts of an intervening galaxy — can completely obscure a background quasar at wavelengths below $(1+z) \times 912 \text{ Å}$, rendering the helium 304 Å transition undetectable. In a previous paper⁵, Møller and Jakobsen calculated that a typical high-redshift quasar lies behind six such sources of neutral hydrogen and that no more than a few per cent of all high-redshift quasars are unobscured at ultraviolet wavelengths. The key to detecting an ionized intergalactic medium is to find a rare high-redshift quasar that is unobscured by foreground neutral hydrogen.

Jakobsen and colleagues began their search for such a quasar in 1990, using the Faint Object Camera on the HST to record far-ultraviolet spectra of 25 high-redshift quasars. The quasar Q0302-003 emerged as the prime candidate, based on a spectrum obtained on 4 December 1992. But at the time, the HST suffered from its well-publicized problem of spherical aberration, and the spectrum of Q0302-003 was suggestive but inconclusive. Better data were clearly needed, so Jakobsen and colleagues reobserved Q0302-003 on 24 January 1994, soon after the COSTAR corrective optics were installed on the HST by the crew of the Space Shuttle *Endeavor*.

This time the result was outstanding. The ultraviolet spectrum of Q0302-003 shows a strong signal that can be traced from 2,000 Å to 1,300 Å. But the spectrum exhibits an abrupt discontinuity near 1,300 Å and shows no signal below 1,300 Å. The discontinuity occurs at just the wavelength predicted for the helium 304 Å transition at the redshift $z = 3.286$ of Q0302-003. The close agreement between the observed and predicted wavelengths of the discontinuity and the absence of signal below the discontinuity strongly suggest that the absorption feature is produced by smoothly distributed singly ionized helium — Jakobsen and colleagues appear to have detected the long-sought signature of the diffuse intergalactic medium.

The case is not airtight, however. One possibility is that the absorption feature is produced by Lyman continuum absorption from a foreground source of neutral

hydrogen at a redshift near $z = 0.42$. But the probability of such a chance coincidence is slight, and ground-based observations of Q0302-003 show no evidence to support this. Another possibility is that it is produced by the many discrete 'Lyman- α forest' clouds that make the thick 'forest' of Lyman- α absorption lines seen at high redshifts. But Jakobsen and colleagues argue that the known population of Lyman- α forest clouds could not produce the observed absorption feature unless the ratio of singly ionized helium to neutral hydrogen in these clouds were implausibly large. An as yet unidentified population of extremely tenuous Lyman- α forest clouds could produce the absorption feature, but such tenuous clouds would probably fill much of intergalactic space and might themselves constitute the intergalactic medium. So the odds are that the observation is just what it appears to be — the discovery of the diffuse intergalactic medium.

What is its significance? Plenty. First, it culminates a long and difficult search and in the process opens up to study the very material out of which galaxies must have formed. Second, it provides the first direct evidence that the intergalactic medium is a highly ionized plasma of hydrogen and helium and challenges astronomers to find the sources of ionization. Third, it supports the Big Bang model of nucleosynthesis, which predicts that light elements like helium were produced in the very early Universe and should be seen at the very largest redshifts.

What next? One possibility is to move to lower redshifts and shorter ultraviolet wavelengths, where the problem of foreground neutral hydrogen is less severe. This will require the next generation of far-ultraviolet satellite telescopes. But neutral hydrogen in our own Galaxy obscures the extragalactic Universe at wavelengths below 912 Å, so the search for the helium 304 Å transition will be forever limited to redshifts satisfying $(1+z) \times 304 \text{ Å} > 912 \text{ Å}$, or $z > 2$. Paradoxically, it seems that we are destined to know more about the intergalactic medium in the distant reaches of the Universe than the intergalactic medium in our own backyard, at least until another probe of the ionized intergalactic medium is found. □

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A snapshot of whole mantle flow

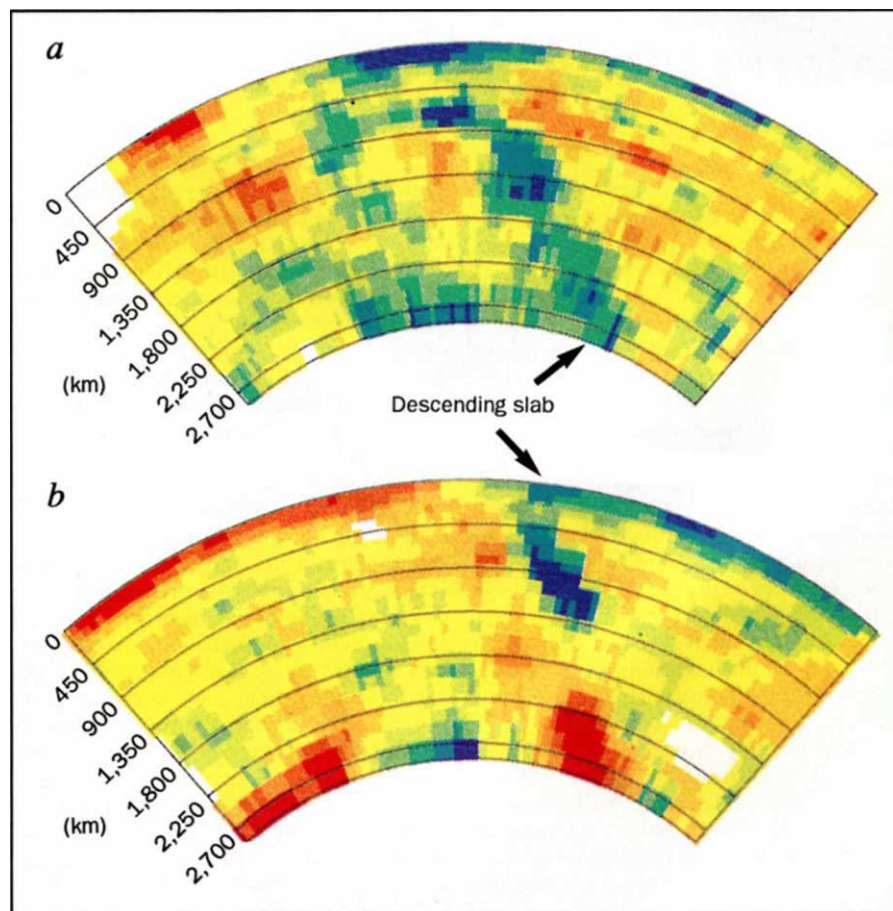
John E. Vidale

In last month's issue of the *Journal of Geophysical Research*, Stephen Grand shows that a slab of the oceanic lithosphere beneath the west coast of America is sinking, almost without check, through the Earth's upper mantle and down into the lower mantle¹. His results add a new twist to a debate that has long exercised geoscientists. Convective flow in the mantle allows heat from the Earth's interior to rise to the surface, whence it radiates into space. But is the pattern of convection a simple overturning of the entire mantle, or does it occur in two or more stratified layers²?

For three decades the debate has been a lively one. Opinions have tended to be polarized. On the one hand, geochemists have argued that the degree of chemical enrichment in the crust indicates that only a portion of the mantle has been correspondingly depleted and that differences between hotspot and ridge composi-

tions suggest the mantle has not been thoroughly mixed; mineral physicists have argued that the lower mantle may be too dense to have the same composition as the upper mantle, and thus that convection must be stratified. On the other hand, seismologists and geodynamicists have uncovered conflicting clues, but most favour at least some penetration of the subducting slabs into the mid-mantle, perhaps with ponding at the bottom of the upper mantle punctuated by occasional flushes into the lower mantle. However, before the work of Grand, the lack of high-resolution models of the lowermost mantle has precluded demonstration that there is flow across the entire mantle.

Grand's new seismological results¹ provide the clearest picture yet of cold material downwelling through the lower mantle. As seismic waves travel faster through cold rock than warm rock, the signature of such downwellings is a slight



Two cross-sections from the surface of the Earth to the core-mantle boundary, showing seismic shear-wave velocity¹. Blue indicates faster velocity, red slower velocity. The peak-to-peak variation shown in the lower mantle is 1 per cent. *a*, A cross-section beneath North America that passes through Portland, Oregon. The fairly continuous fast anomaly extending down to the core is seen where subduction has been rapid for the past 150 million years. *b*, A cross-section beneath South America that passes through Peru. A distinct lower limit to the fast anomaly is seen where subduction has been rapid for only the past 50 million years.

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speeding up of the seismic waves passing through it. These travel-time signals from the lower mantle have proved difficult to interpret because of overprinting by the larger variations in the uppermost 700 kilometres of the mantle. But Grand has carefully incorporated the seismic raypaths that turn in the upper mantle, which are usually omitted or treated in simpler ways. By this process, and the inclusion of waveform data from the numerous analog seismometers that operated in the 1960s and 1970s, he has been able to determine the three-dimensional upper-mantle structure, which is interesting in its own right, and to isolate the lower-mantle structure.

An extensive slab of rock that is seismically fast and therefore cold is seen sinking in the lower mantle beneath the subduction zones spanning the west coast of the Americas (see figure). The faster material is visible from the top of the lower mantle, near 800 km depth, to the base of the mantle. The subduction zones known to be present in the upper mantle are not clear, probably because they are masked by other mechanisms that cause strong velocity variation in the upper mantle, and also because the subducting slab is so narrow in the upper mantle.

The downwelling crosses the depth of 660 km, where there is a phase transition that was widely suspected to inhibit mantle flow^{3,4}. There is no sign of cold material ponding at the base of the upper mantle over large areas (more than 1,000 km), as has been suggested from numerical simulations that are marked by episodic flushing of subducted material through the lower mantle^{5,6}. The continuity of the descending sheet instead suggests steady flow in this region, although studies in the western Pacific^{7,8} have found extensive volumes with fast seismic velocities beneath a few subduction zones, interpreted as ponding of slabs at the base of the upper mantle.

In Grand's work, the width and flow rate of the downwelling can be inferred to differ between the upper and lower mantles, whereas the initial seismic measurements that indicated slab penetration into

Physics for macaques

IMAGE
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REASONS

THIS is Tokei, a female Japanese macaque, at work retrieving an apple from the middle of a transparent tube by throwing a stone at it (a, Tokei throws stone; b, stone hits apple; c, Tokei moves to the other end of the tube and, d, collects the apple). During an investigation of macaque tool use at Jigokudani Monkey Park, Japan, Tokei emerged as a remarkable individual in her inventiveness in finding ways to extract the fruit from the tube. The study, described by E. Tokida *et al.* in *Animal Behaviour* (47, 1023–1030; 1994), started with the macaques being offered a pre-positioned hooked stick with which to pull out the apple. The retrieval options involving sticks were gradually increased in difficulty, culminating in Tokei's use of a natural shrub root she shortened for the purpose. At about the same time, the stone-throwing technique was discovered and eventually four macaques picked it up. The drawback was that the apple could be filched by a rival diner if the stone were thrown too hard. Tokida *et al.* found that Tokei threw stones with less force when other macaques were close to the other end of the tube — giving her more of a chance to see off the competition — than when they weren't; hence their inference that macaques have an appreciation of the principle of the conservation of momentum in collision. Their final observation lends itself to all manner of anthropomorphic speculation. Only Tokei brought her infants to the tube, pushed them in and got them to fetch the apple for her.

Tim Lincoln

Courtesy Academic Press / I. Tanaka

the lower mantle⁹ could not resolve such differences. The rate of sinking is inferred by comparing the depth of the lower edge of the sheet to the time at which subduction was initiated. Beneath the segment of the South American subduction zone where the rapid subduction commenced 50 million years ago, the sheet disappears at a depth of 1,300 km, so the rate of sinking in the lower mantle is about 1 cm per year. Beneath North America, subduction has been continuous and rapid throughout the past 150 million years, and the lower-mantle anomaly extends to the core. The subducting material sinks about five times more slowly and thus spreads to form a broader downwelling in the lower mantle than the upper mantle.

A similar result has come from modelling the geoid¹⁰. Ponding scenarios, on the other hand, would predict a much wider zone of seismically fast material concentrated near 660 km depth. The measured width of the downwelling under the Americas, 500 to 1,000 km, is in accord

with previous local estimates¹¹. The differences between upper- and lower-mantle downwelling have been linked to possible differences in the viscosity¹².

The extensive slab of descending material beneath the Americas implies that one convection cell spans the entire mantle, at least in this region. As the best image yet of lower-mantle structures on a scale of 300–1,000 km, this observation of large amounts of mass flux between the upper and lower mantle causes great difficulty for those who would argue that the mantle has not thoroughly mixed since its formation billions of years ago.

The debate is far from over. But Grand's model and interpretation of the fate of slabs descending into the lower mantle looks likely to guide our hypotheses for some years to come. □

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Digitizing the skies

S. George Djorgovski

CHARTING the skies is the most ancient of astronomical pursuits. Now, in the spirit of the age, astronomers are busily paving their own information superhighways, and compiling digital road maps to guide them along. This happy activity was very apparent at a recent meeting*, where we learned that numerous ambitious projects are being planned or are underway to map the sky at wavelengths ranging from the radio to the X-ray. Each digital survey will produce a census of all sources detected down to its appropriate flux limit, numbering thousands, millions or even billions.

This is all to the good. Traditionally, astronomers have dealt with individual interesting objects, or small classes of these. What is afoot here is both qualitatively and quantitatively different: it is a prologue to systematic studies of the entire sky, and entire classes of objects, with homogeneous data: for example, all galaxies detected down to a given flux limit in a given bandpass. To be sure, sky surveys, even digital ones, have been done over portions of the sky in the past, but not with the scope of the projects now being hatched. The forthcoming rich harvest of information will enable astronomers to ask, perchance to answer, whole new sets of questions. There is also strength in numbers: for example, given a well calibrated and well understood catalogue of a billion or so stars, one can use the statistics reliably to check on the finer points of galactic structure models.

Sky mapping in visible light remains the staple of cosmography. Photographic-plate atlases originating from the Schmidt telescopes in both hemispheres are now being digitized by several groups, not the least of which is the Automatic Plate Measuring Machine team, which hosted the meeting (R. Humphreys, Univ. of Minnesota). Extremely useful work is being done at the Space Telescope Science Institute (STScI), stemming from their need to compile a guide-star catalogue for the Hubble Space Telescope. Their plate scans of both the northern and the southern sky surveys are now being released to the general community as compressed images on CD-ROM disks (B. Lasker, STScI). These digital versions of the sky

survey prints, plates and films so familiar to the present generations of astronomers will extend the scientific lifetime of the photographic sky surveys well into the computer era.

The work does not end with the digitization of the plates, which produces about a terabyte's worth of pixels per sky per bandpass. Most groups are busy extract-



One 'microsky' — the image above shows a random sample of the sky, digitized from a photographic plate from the Second Palomar Observatory Sky Survey. The field is about 12 arcmin across (0.2 degree), and the total area is about one millionth of the whole sky. About 100 galaxies and 200 stars are visible down to the sensitivity limit of the plate (about 22 mag). (Courtesy of Palomar Observatory, California Institute of Technology.)

ing catalogues of stars or galaxies from the plate scans for use in a variety of scientific endeavours. One of the most ambitious is surely the Precision Measurement Machine project at the US Naval Observatory's Flagstaff Station (US NOFS), which aims to scan both the first and the second Palomar Observatory Sky Surveys, in both blue- and red-sensitive plates (D. Monet, US NOFS). The principal result will be accurate positions and proper motions of many millions of stars in the northern sky, essential data for stellar and galactic astronomy.

Our group at the California Institute of Technology and Jet Propulsion Laboratory is also processing the latest of the STScI plate-scanning efforts, a digital version of the Second Palomar Sky Survey. We hope to produce a calibrated catalogue of over 50 million galaxies and over a billion stars, in three colours, down to

the survey limits (roughly equivalent to the twenty-second magnitude in the blue). The first results from galaxy counts and clustering studies indicate higher internal consistency of the data than had been achieved with a comparable photographic plate material. The catalogue should become available in instalments starting about a year from now.

These digitization efforts are giving a second lease of life to astronomical photography, an otherwise moribund field (the artistry of David Malin notwithstanding). But the future clearly lies in the fully digital sky surveys, using modern charge-coupled device (CCD) detectors. Foremost of these is the Sloan Digital Sky Survey (SDSS) being prepared by a large consortium of institutions (R. Kron, Univ. of Chicago). Their plan is to obtain images of the sky at high galactic latitudes in five photometric bands, and then to obtain spectra of about a million galaxies and quasars. A 2.5-m telescope is being built for this purpose at the Apache Point observatory in New Mexico, equipped with a formidable array of 30 CCDs with 2,048² pixels each. They hope to start in 1996, and finish the survey in about five years, producing some 20 terabytes of data. Their goals include studies of the large-scale structure of the Universe, galactic structure and searches for new quasars.

In the past, whenever a new wavelength window was opened, a sky survey was soon forthcoming just to see what was out there; recall, for example, the early surveys of the radio sky done in Cambridge, or the IRAS satellite coverage of the far-infrared sky. Although we now have some idea of the appearance of the sky at

most wavelengths from γ -rays to radio waves, the details are often sketchy. Most of the science from such efforts so far has been produced by cross-correlating the sources detected in different wavebands. Optical identifications of radio or X-ray sources are considered necessary before their nature or physical properties can be determined, and observations at different wavelengths can draw attention to an otherwise nondescript optical object; this is, after all, how quasars were discovered.

One of the most yawning gaps in our multiwavelength coverage of the sky is in the near-infrared. The long-awaited Two-Micron All Sky Survey (TMASS), again planned by a consortium of institutions, is expected to start in 1997 (S. Kleinmann, Univ. of Massachusetts). Its European, southern-sky counterpart, the Deep Near Infrared Survey (DENIS) may start as early as 1995. The former is the more

*184th Meeting of the American Astronomical Society, Minneapolis, Minnesota, 31 May–2 June 1994.

ambitious in scope and coverage, but many of the parameters of the two surveys are comparable. The TMASS will reach limiting magnitudes around 15 in the J, H and K infrared bands, and will cover the entire sky using pixels 2 arcsec across. Two dedicated 1.3-m telescopes are planned for the survey, one for each hemisphere. These data will nicely complement the optical and far-infrared surveys now under way. Their uses will range from exploring obscured regions of the Galaxy to searching for brown dwarfs, and may well lead to the discovery of new types of active galaxies.

Radioastronomers are not slouching either. Two sky surveys are now starting at the Very Large Array in New Mexico. One is led by J. Condon of the National Radio Astronomy Observatory (NRAO), using the array in a lower angular resolution than usual to match, for instance, the resolution of the IRAS data; it will cover most of the sky visible from the array. The other, aiming to take faint images of the radio sky at 20 cm (and therefore named FIRST) will reach comparable flux limits, about 1 mJy, at an angular resolution of 5 arcsec (D. Helfand, Columbia Univ.; R. Becker, Univ. of California at Davis). It is expected to cover about 10^4 square degrees at high galactic latitudes, essentially the area covered by the SDSS, and to yield about a million radio sources, most of them presumably distant radio galaxies and quasars.

These are vast amounts of information. Their processing and scientific analysis call for a new generation of astronomical software, to detect, measure and classify the sources — distinguishing automatically between stars and galaxies, for instance — and then to maintain and access the resulting databases efficiently. Novel techniques such as neural nets are being drafted in to help automatically classify objects, including such details as the morphological types of brighter galaxies (S. Odewahn, Univ. of Minnesota). Other ideas abound, and astronomers are likely to see more machine-learning and machine-assisted discovery techniques used for the exploration of huge parameter spaces.

All this is hard and sometimes thankless work. But this is the information infrastructure for the new astronomy. To quote one of participants at the meeting (M. Kurtz, Harvard-Smithsonian Center for Astrophysics), the collections “form the foundation for the ongoing research and discovery which characterize astronomy”. Who knows what surprises could lurk in the gigasource databases? □

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Cognitive maps in infants?

John O'Keefe

As infants begin to crawl and then walk, they need navigational strategies to guide their movements no less than do foraging rats and homing pigeons. Are the strategies they use, and the brain mechanisms that subserve them, the same as those in lower vertebrates? In particular, is there a specialized neuromodule dedicated to spatial localization and navigation which is conserved across species? These are just some of the questions provoked by the report on page 57 of this issue by Hermer and Spelke¹.

In a series of experiments modelled on ones originally performed on rats^{2,3}, Hermer and Spelke show that, following disorientation by gentle rotation, infants use the shape of a rectangular chamber to locate a toy in one of the corners, but do not make use of additional information such as the colour of one wall or the location of salient objects. As one might expect, adults in the same situation do make use of the information provided by a distinctive wall colour to improve their spatial discrimination. This finding supports the idea that geometric information about the shape of an environment is processed separately from the colour of the walls or the shape of objects located in that environment, at least in infants of 18–24 months of age. In the authors' terms, the information is encapsulated in a geometric module. Is the perception of the surrounding environment based on a different neuromodule from the perception of objects, faces or colours? Is it perhaps carried out in a specific part of the brain that accepts or acts on very specific types of information?

The animal work on which the Hermer and Spelke study is modelled is part of a larger body of work on the types of cues and navigational strategies used by animals in different environments. In symmetrically shaped environments such as the Olton radial maze⁴ or the Morris water maze⁵, it is clear that cues outside the maze itself (if they can be perceived by the animal) form an important source of input to the animal's spatial representations. If animals are prevented from perceiving these distant extra-maze cues, for example through the use of enclosed boxes or mazes, the shape of the enclosure itself dominates, particularly if the animal is disoriented so that it cannot carry over its general orientation relative to the external environmental framework into the testing apparatus. This is the paradigm that forms the model for the present experiment. As the authors note, under these circumstances rats and infants fail to use proximal cues other than room shape to locate a goal, and presumably

themselves, within the environment.

Work on the activity of cells in the mammalian brain may help to explain their results. Spatially coded cells have been found in several different brain regions, including parietal lobes⁶, prefrontal cortex⁷ and superior colliculus^{8,9}, but these all appear to code the locations of objects within coordinate frameworks centred on the retina, head or body. Cells in the hippocampus, on the other hand, appear to code for location in an allocentric or environmentally centred framework¹⁰. These place cells become selectively active when the animal visits a particular part of an environment and appear to mirror the animal's use of spatial stimuli: on elevated mazes such as those devised by the late David Olton, they take their location from the distal cues, whereas in enclosed boxes, where distant information is reduced or excluded, they follow the internal enclosure-derived cues¹¹. In these latter environments, place cells are particularly sensitive to the shape of the enclosure, differentiating, for example, between square-shaped boxes and cylinders which are identical in all other respects¹². This discrimination would appear to be part of the computation made by the hippocampal cells themselves, as cells in the entorhinal cortex, the area that acts as a conduit for most of the exteroceptive information to the hippocampus, are not sensitive to environmental shape¹³.

Animals are sensitive not only to places in an environment, but to directions as well. Cells in another part of the hippocampal formation, the presubiculum, become active whenever the rat points in a particular direction relative to the

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environment frame, irrespective of its location within the environment¹⁴. These cells use the local enclosure-based cues as a reference as well, but they may also have vestibular inputs that enable them to update constantly their estimate of the current heading direction on the basis of the animal's movements. It is these cells and this inertial guidance system that is most likely to be disrupted by the disorienting strategies (for example, rotation with eyes closed) used in the Hermer and Spelke experiments. Rotating a rat in a similar fashion before it is put into an enclosed box results in an unpredictable rotation of both the direction and place cells relative to the environment. The two sets of signals maintain coherence, however, suggesting that the polarization of an environment might be

given by the direction system¹⁵.

The results of Hermer and Spelke suggest commonalities between the spatial systems of rats and human infants. Their experiments were not designed to distinguish between place and direction strategies, and it would be interesting to see which of the systems their infants were using. It is possible that they cannot use colour or objects to reset the direction sense until much later in life. General frameworks for the understanding of navigation in humans and animals¹⁶ will have to take these developments into account. □

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EMBRYOLOGY

On the loss of Mos

George F. Vande Woude

It is always good news when the phenotype of a germ-line knockout mouse turns out as expected. On pages 65 and 68 of this issue^{1,2}, two groups independently show that the fertility of female mice is severely impaired when they lack the *c-mos* proto-oncogene, whose product Mos has long been recognized as a regulator of oocyte maturation.

The *c-mos* gene was the first proto-oncogene to be molecularly cloned and have its oncogene activity demonstrated³; its product, Mos, is a serine/threonine kinase. Surprisingly, given its activity as an oncoprotein, Mos is not found in somatic cells but only in meiotic germ cells, and studies in *Xenopus* and mouse oocytes have identified Mos as a key regulator of meiosis (for a review, see ref. 4). The latest experiments from Colledge *et al.*¹ and Hashimoto *et al.*² reported in this issue use targeted disruption of the *c-mos* gene in embryonic stem cells to generate *mos*^{-/-} mice. Male mice lacking *c-mos* are viable and fully fertile, whereas female mice are viable but display reduced fertility as a result of the parthenogenetic activation of mature oocytes. These results show that Mos is not required for male gametogenesis, nor is it required (or is redundant) for the development and growth of the somatic lineages. They also indicate that *c-mos* is necessary for metaphase arrest of oocytes at meiosis stage II. Other notable features of the *mos*-deficient mice are the parthenogenetic activation of their eggs and the development of ovarian cysts and teratomas in the females.

Oocyte maturation requires maturation promoting factor (MPF) — a complex of cyclin B and the cyclin-dependent kinase

p34^{cdc2}. Mos has often been implicated as a regulator of MPF⁴. In *Xenopus*, Mos is required for the reinitiation of meiotic maturation, the activation of MPF, for germinal vesicle breakdown and for the formation of the first polar body, as well as for reactivation of MPF during metaphase II (ref. 4). It is also an active component of cytosolic factor (CSF), believed to be responsible for the arrest of oocyte maturation at metaphase II, the unfertilized egg stage of development⁵. Of all these activities in *Xenopus* oocytes, the maturing oocytes of *mos*^{-/-} mice appear to be deficient in only the CSF-like activity^{1,2} and show a slower rate of meiosis after germinal vesicle breakdown².

These studies show that meiosis I in mice is significantly different from that in *Xenopus*, a not-so-surprising result given that, for instance, amphibians produce large numbers of eggs synchronously. In contrast to mice, the *Xenopus* G2/M transition into meiosis I and subsequent germinal vesicle breakdown are sensitive to protein-synthesis inhibitors and Mos is necessary and sufficient for meiosis I (ref. 4). In other mammals (pigs, sheep, cows)⁶, germinal vesicle breakdown is also blocked by protein-synthesis inhibitors. The requirement for protein synthesis during meiosis I in other mammals may indicate a requirement for Mos, although the results of Colledge *et al.*¹ and Hashimoto *et al.*² raise the possibility that meiosis I could be regulated by the *de novo* synthesis of other components of the meiotic pathway, such as cyclins. Mouse Mos, compared to *Xenopus* Mos, is much more active as an oncoprotein and as an inducer of *Xenopus* oocyte maturation

and blastomere CSF arrest⁷. It is surprising, therefore, as the two new studies reveal^{1,2}, that Mos has a lesser role in murine meiotic maturation.

What is the requirement for Mos during meiosis? In *Xenopus*, Mos not only triggers MPF activation, but is also a potent activator of the MAP kinase pathway⁴. In addition, MAP kinase has been implicated in CSF activity⁸. How are these activities related to the meiotic process? Mos kinase is suspected to be involved in the reorganization of microtubules that leads to the formation of the spindle and spindle pole^{4,9}, and it is a component of CSF, which contributes to the stabilization of the spindle at metaphase II (ref. 10). Consistent with the role of Mos in activating MAP kinase, MAP kinase induces M-phase microtubule reorganization *in vitro*¹¹ and is localized to the microtubule-organizing centres in mouse oocytes¹². In mice, the critical activity of Mos appears to be at metaphase II of meiosis^{1,2}. It is possible that the *mos*^{-/-} oocytes at metaphase II are also deficient in MAP kinase activity and are therefore unable to stabilize the spindle at this stage.

Constitutively activated MAP kinase does not initiate oocyte maturation⁸, implying that, in *Xenopus*, Mos must activate MPF through a pathway distinct from MAP kinase activation¹³. What about the slower rate of meiosis after germinal vesicle breakdown, as described by Hashimoto *et al.*²? One must conclude from the *mos*^{-/-} mice that MAP kinase either does not participate in the initiation of mouse oocyte maturation or, more likely, that a redundant activity, such as the Ras/Raf pathway, compensates for Mos. The slower rate of meiosis after germinal vesicle breakdown could indicate that Mos kinase normally enhances this activity.

When Mos is depleted during meiosis in *Xenopus*, oocytes fail to re-enter meiosis II, the nuclei re-form, and DNA synthesis is initiated¹⁴. As Mos both prevents DNA synthesis and induces cell-cycle arrest at metaphase II, *mos* could be considered as a meiotic tumour-suppressor gene. Consistent with this idea, the loss of Mos leads

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to parthenogenesis^{1,2} and to the development of teratomas². But when *mos* is inappropriately expressed in somatic cells, it does not have either of these effects; the only major function in common with oocytes is the serum-independent activation of MAP kinase (K. Fukasawa *et al.*, personal communication). In this regard, *Mos* is like other

dominantly acting oncogenes and, as proposed⁴, may be imposing a meiotic-like phenotype on all stages of the somatic cell cycle. □

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ENGINEERING

Acoustic micromachines

David E. Newland

MORE than 300 million microphones are sold every year, with telephones, cassette recorders, camcorders, personal computers and hearing aids being some of the biggest markets. One of the highlights of an acoustics conference at MIT recently* was a paper giving technical details of new silicon semiconductor microphones no larger than a pinhead (G. Sessler, Darmstadt Technical University). These miniature devices are expected to find many applications.

Since the early 1980s, engineers have been developing silicon chip microphones using the methods of semiconductor technology in which anisotropic etching allows three-dimensional structures to be produced on silicon wafers. Membranes of silicon as thin as a fraction of a micrometre (less than one millionth of a metre) can be etched from solid and used to provide the flexible diaphragm required for a microphone. Pressure changes due to sound waves cause this tiny diaphragm to deflect and alter the internal capacitance of a device which is only a millimetre or two in size.

The diaphragm is one plate of a capacitor. Typically, it may be about 1 mm² in area and the air gap which separates it from its backplate about 2 µm wide (Fig. 1). The trick is to obtain good acoustic properties in something so small. Holes in the backplate allow the air-gap damping to be kept small and the effect of stray capacitance is reduced by incorporating p-n junctions at the edges of the backplate. Good sensitivity with a flat response up to 10 kHz can be obtained and with noise levels comparable to conventional condenser microphones.

Micromachining methods can also be used to produce piezoelectric microphones in which the membrane is of a piezoelectric material that generates an output voltage when it is deflected. In one design, a layer of piezo-polymer about 0.5 µm thick is evaporated onto a silicon diaphragm which is itself only about 1 µm thick. As the strain in the piezoelectric

layer is of opposite sign at the periphery of the diaphragm from that at its centre, separate electrodes have to be attached to the inner and outer regions and connected in series to ensure that the piezoelectric signals of both regions are added in phase. Other miniature microphones use piezoresistive elements to provide the sensor. Typically, four polysilicon resistors are positioned on a 1 mm² silicon

can be obtained, although at present the noise level is relatively high.

Apart from being smaller than conventional devices, silicon microphones have other advantages. The most important is that they can be manufactured in bulk using the techniques of the semiconductor industry, which means that they are going to be cheap and reliable. Also, they can be produced with appropriate electronics on the same chip, and their mechanical and response properties are likely to be better than conventional microphones. For example, because the tiny silicon diaphragm can be so light while still retaining intrinsic tension, its natural frequency is high, so its sensitivity to vibration is much reduced and it does not pick up extraneous signals from vibration sources.

Two 'hot topics' identified at the conference were solid-state devices and hearing aids (I. Busch-Vishniac, Univ. Texas at Austin). In addition to microphones, some of the solid-state devices under discussion were underwater hydrophones, cantilever-beam accelerometers (already being introduced commercially for auto-

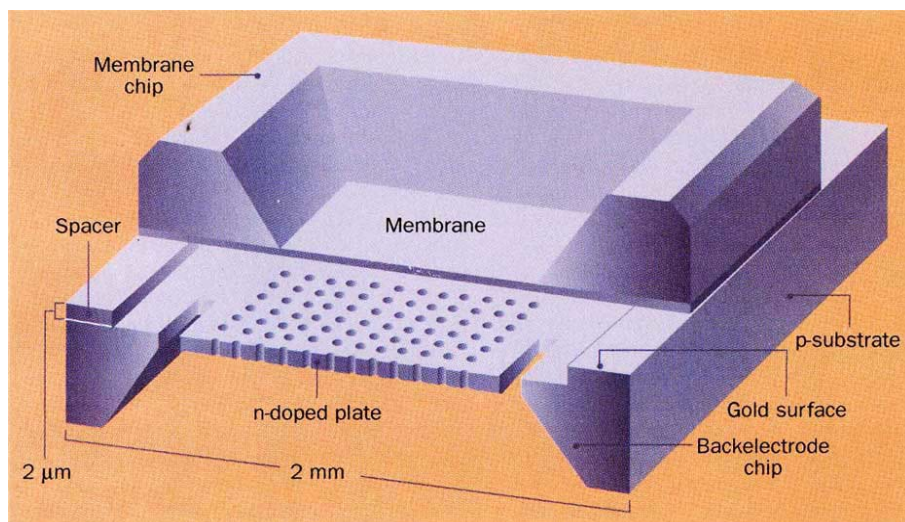


FIG. 1 Simplified sketch of the plates of a two-chip silicon condenser microphone. (Based on a diagram provided by G. Sessler.)

diaphragm 1 µm thick. Two are located in the centre region and two at the edges of the membrane and, like conventional strain gauges, they are connected in a Wheatstone bridge to measure the incremental change in resistance that deflection of the diaphragm generates.

For instruments developed to date, silicon condenser microphones have the highest sensitivity and piezoelectric microphones the highest frequency response (first resonance frequencies as high as 45 kHz have been reported). Work is also going on to use sound pressure to modulate the output of a field-effect transistor directly. Movement of the microphone's silicon membrane modulates the gate-channel capacitance and hence the drain current of the transistor. Good sensitivity and a high-frequency response

mobile air bag deployment), miniature mechanical actuators for ciliary motion and miniature valves with vacuum-deposited films for the moving parts. These cover more than acoustic applications and some are already established in the market. The 1995 market for micromachined acceleration sensors for automobile crash detection is predicted to be about 4 million units and expected to grow rapidly. Already, micromachined silicon piezoresistive pressure sensors for engine manifold pressure measurement are made by the Delco subsidiary of General Motors at a rate said to be more than 10 million units a year. At an average price of \$10 per integrated package of transducer and signal-conditioning circuit, it has been estimated that the worldwide market for these devices is worth about \$250 million

*127th Meeting of the Acoustical Society of America, MIT, Cambridge, Massachusetts, 6-10 June 1994.

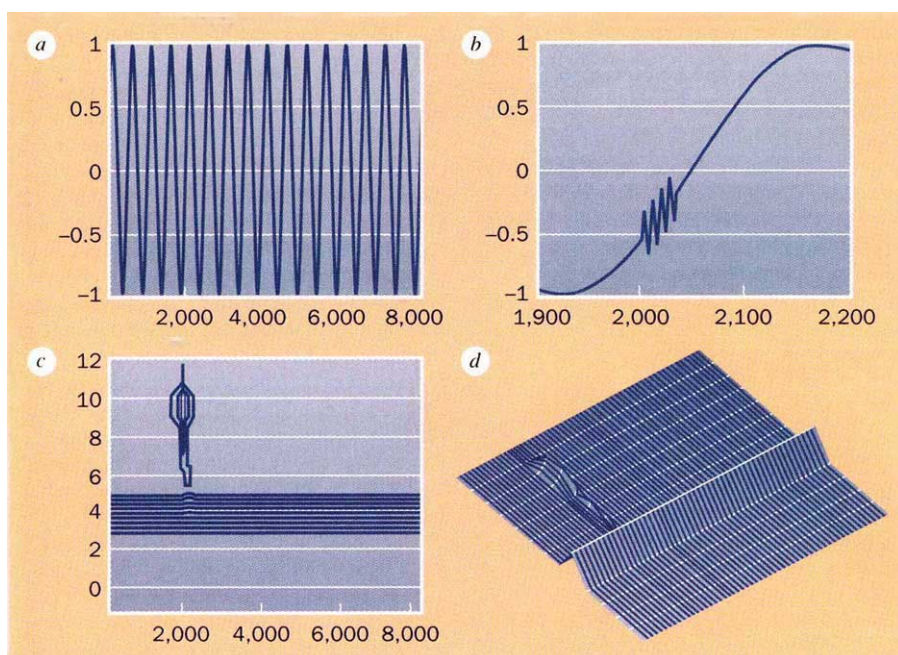


FIG. 2 Wavelet analysis of a periodic signal with a local perturbation. *a*, Sine wave with perturbation; *b*, zoom onto perturbation; *c*, harmonic wavelet map; *d*, mesh plot of wavelet map. The horizontal scales in *a*, *b* and *c* represent time. The vertical scale in *a* and *b* is non-dimensional; in *c* it is frequency.

per annum. So there are enormous opportunities for the new technology.

Hearing aids are likely to be another very important application for silicon micromachines and one that takes advantage of the ability to combine a microphone and its amplifier on the same chip, thereby miniaturizing the whole device. If these chips can carry a miniature speaker to generate the amplified sound, the resulting integrated acoustic machine is likely to transform life for the hard of hearing. The conference heard how advances in signal processing for hearing-aid amplifiers have greatly improved the quality of amplified sound presented to the ear. In addition, clever signal processing of the outputs from arrays of tiny microphones can adapt to changing acoustic environments by focusing on sound sources and attenuating background noise and interference. For those with major hearing impairment requiring cochlear implants, the advantages of microminiaturization are profound.

There are many applications where silicon microtransducers will simply replace conventional transducers. But to an increasing extent the new technology is also developing and extending existing markets. Vibration signature monitoring is an example. By monitoring the vibration of machinery, it is possible to anticipate breakdowns. When gear teeth and bearings wear, they begin to run roughly and generate tell-tale vibrations. If these vibrations can be detected early enough, maintenance work can be planned and costly failures avoided. But conventional data collection and analysis equipment is expensive and this limits its application to high-cost products. In contrast, micro-

machined sensors with on-chip electronics and software will be cheap and many new applications will arise as a result.

For vibration signature monitoring, there typically need to be very many transducers, strategically placed, with fast data collection and processing. Because silicon accelerometers are so small and robust, and because they will be inexpensive, there can indeed be many of them. The data they record have to be digitized, compressed and interpreted fast, which depends on the effectiveness of the computer algorithms used to store, transmit and interpret the vibration patterns that are recorded. New software, using the theory of wavelet analysis, will make it possible both to analyse and compress the data before transmission by on-chip algorithms.

The principle of detection is illustrated in Fig. 2. A harmonic signal has a small local perturbation which shows as a local ridge on the wavelet map. The position and frequency of the perturbation has been identified by the transform. Although the first interest in practical wavelet software derived from the need to obtain good data compression for speech and video transmission, there are likely to be applications in many unrelated fields. The combination of very-large-scale integration technology from the microelectronics industry with smart software and micromachined mechanical sensors offers great scope for health monitoring both of machinery and people. □

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DAEDALUS

Wind surfing

THE kite, that cheerful outdoor toy, is a lifting aerofoil, held and angled in the wind by a tether to the ground. Thanks to modern materials, such as polyester sheet and polyimide fibre, it is now a research tool. Kites can lift useful scientific loads several kilometres into the sky (B. B. Balsley *et al.*, *Nature* 369, 23; 1994). Daedalus is now designing a high-altitude kite that does not need to be tethered to the ground. It exploits the fact that the velocity of the wind varies with height.

Imagine, he says, a kite flying at some high altitude where the wind is blowing strongly. It is tethered, not to the ground, but to another kite flying lower down, where the wind is blowing less strongly, or in a different direction. Each kite would hold the other against its local wind. The lower kite might be inverted, tensioning the connecting tether by means of downward 'lift'; or the tethers of both kites might lift a common payload beneath them. The arrangement would be quite unstable, especially in gusty conditions. To maintain a stable height, it would need a fast-acting on-board computer to control its lifting surfaces.

As a self-contained flying machine, this novel 'bikite' is elegantly simple. It could fly at any height that spanned an adequate difference of wind velocity. It could even steer a course. Just as a sailing ship can travel in any direction by exploiting the difference in velocity between wind and sea, so the bikite could travel by exploiting the velocity difference between two winds.

The bikite should come into its own in that little-explored region between 80 and 120 km up. Too high for aircraft, too low for satellites, it has a fairly stable east-west wind shear of about 2.5 metres per second for every kilometre of altitude. Daedalus is designing huge frail bikites which, released from balloons, will lift instruments and transponders up into this region. With their electronics and control systems powered by small windmills or solar cells, and metallized polymer aerofoils shaped as huge down-gazing microwave aerials, they will stay up indefinitely.

Their main application, of course, will be communication. A set of aerospace bikites, each beating about a fixed point or all blowing endlessly round the globe, would make an ideal communication net. Being much nearer the Earth than a geosynchronous satellite, they could talk and listen to any transceiver with a crude nondirectional aerial, and relay signals to other bikites in the net. Cheap, simple and easily replaced, they would be ideal for the proposed worldwide cellular telephone network.

David Jones

Mate competition in butterflies

SIR — Pupal mating occurs in several insect orders (see ref. 1 for review) and 42% of *Heliconius*², but in only one butterfly outside this genus³; thus it represents one of the most extreme forms of male mating behaviour in the Lepidoptera. Male *H. hewitsoni* search the larval host plant, *Passiflora pitiaria*, for female pupae, inspect these pupae regularly, then compete with each other for a position on a pupa and for access to the female as she ecloses (see figure). Two distinct selective events can be identified within inter-male competition in this system: (1) competition for a position on a pupa; and (2) competition for mating once in position.

We analysed the outcome of male contests for mates in 27 pupal mating events that occurred under natural conditions during 1979–83. A total of 235 males attended the events, with an average of 8.7 males per event ($2-24 \pm 0.9$ s.e.) and 4.9 competing males per female pupa ($1.3-24 \pm 0.9$ s.e.). Attendant males had been previously marked, measured and numbered on each hindwing. Wing and body length were measured to the nearest millimetre, and wing wear, a rough indicator of age, was scored on a scale of 1–6. All

measurements were standardized within each mating event to a mean of zero and a variance of one (z-scores; ref. 4).

Size was important in determining the outcome of male contests in both selective events, whereas wing wear was never a predictor of male success. Selection favours large males in competition for

males from landing by shielding the pupa with their wings (see figure). In contrast, small, flexible abdomens that can be inserted further under the pupal skin should confer an advantage in subsequent competition to clasp the female. The selection gradients⁶ calculated for male traits are consistent with these interpretations: large wings are favoured in the first selective event, with body length affected as a correlated response, and small bodies are

STANDARDIZED SELECTION GRADIENTS

	Wing length(mm)	Body length(mm)	Condition	Total R^2
Sitting	0.44 (0.23)	0.03 (0.23)	-0.04 (0.12)	0.10 (1.4)
Mating once seated	0.15 (0.85)	-0.41 (0.83)	-0.22 (0.43)	0.01 (1.6)

Selection gradients are the partial regression coefficients of a multiple regression of relative fitness on the three characters. Relative fitness was calculated as male absolute fitness (0 or 1) divided by the mean fitness of males attending the same event. Separate multiple regressions were calculated for sitting and mating. Significance levels are omitted because the data, even when transformed, violate the assumption of normality necessary for the calculation of meaningful *F*-ratios and *t*-statistics. Numbers in parentheses, standard errors.

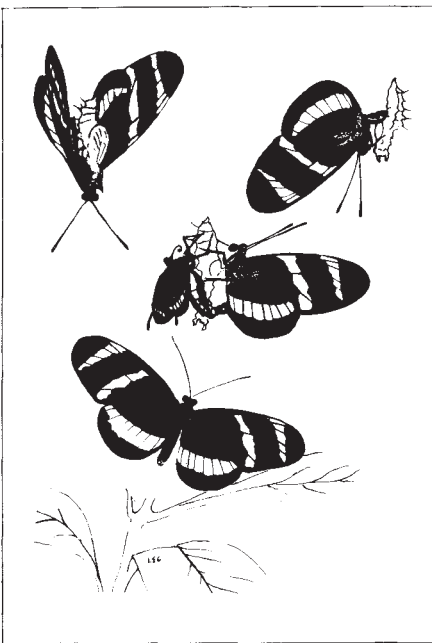
positions on pupae: sitting males were significantly larger in both wing and body length than males that failed to sit (Mann-Whitney *U*-test: $P < 0.001$, $P = 0.002$, $n = 214$). Size does not affect a male's probability of inserting his abdomen once seated (Mann-Whitney *U*-test: $P = 0.17$, 0.90 , $n = 95$, 94), but once a male has achieved a position on a pupa and inserted his abdomen, selection favours small males: males that mated were significantly smaller in wing and body length than unmated males (Mann-Whitney *U*-test: $P = 0.03$, 0.02 , $n = 52$). An effect of size on competitive ability has often been proposed for butterflies and has recently been demonstrated⁵, but until now has not been shown directly to affect the mating success of males.

The effect of size is obscured if the two selective events are considered together, as the opposing selective forces acting on wing and body length interact to cancel each other. Of all males attending mating events, no size difference was detected between mated and unmated males (Mann-Whitney *U*-test: $P = 0.15$, 0.41 , $n = 214$). Such opposing forces have the potential to lead to either stabilizing selection or no selection, depending on their effect on trait variance. Because z-scores reflect trait divergence from a mean of zero, stabilizing selection would be indicated if the squared z-scores of unmated males were significantly larger than those of mated males. A comparison of the z^2 -scores of mated and unmated males revealed no significant net selection on either trait (Mann-Whitney *U*-test: $P = 0.15$, 0.15 , $n = 214$).

Because wing and body lengths are highly correlated ($r = 0.82$), it is possible that one is the target of selection while the other is acted on indirectly. Behavioural observations suggest that such an interaction exists. In competition for positions on pupae, sitting males prevent other

favoured in the second event, with wing length indirectly affected (see table).

Although there is no detectable net selection acting on either wing or body length, males with relatively longer wings and shorter bodies should be favoured by selection. Unfortunately the variance in the wing:body ratio was too low (coefficient of variation = 4.13, $n = 213$) to detect such selection acting on this population. However, similar selective pressures should act on all pupal mating *Heliconius* butterflies, suggesting the possibility of allometric evolution due to pupal mating. Although this hypothesis cannot be tested directly because pupal mating species form a monophyletic group⁷, the hypothesis would be rejected if no significant difference existed between the wing length:body length ratios of pupal and non-pupal mating species. We compared the wing:body length ratios of the three pupal mating species with those of the four non-pupal mating *Heliconius* spp. found at the study site. Although pupal and non-pupal mated did not differ in mean wing length or body length (Mann-Whitney *U*-test, $P = 0.29$, 0.08 respectively), males of pupal mating species indeed had significantly larger wing:body ratios than non-pupal mating species (Mann-Whitney *U*-test, $P = 0.03$). This suggests that selection is alternately targeting wing



Stages in a pupal mating event in *H. hewitsoni*. Flying males (bottom) compete for a position on female pupae and harass males already sitting (top left). Several males may sit on a pupa simultaneously, while others circle the pupa, attempting to land. Males are often displaced during their tenure on a pupa, and may be found lying exhausted or dead on the ground. Prior to female emergence one or two sitting males will puncture the pupal cuticle, inserting up to three abdominal segments under the pupal skin (top right). Mating takes place as the female begins to eclose (centre), and females mate only once.

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length and body length in male competition for mates, and that this may have led to the evolution of higher allometric ratios in pupal mating species.

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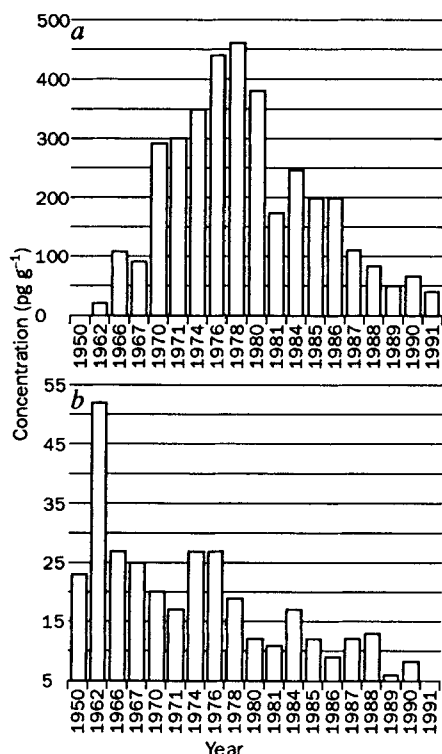
Organolead in wine

SIR — Reports on the use of the polar ice-cap archives to trace global atmospheric pollution by lead^{1,2} stimulated our interest in the use of old wine vintages for a similar purpose (but on a local scale). Toxic effects of lead in wine are well known³, but hardly any attention has hitherto been paid to organically bound lead, despite its far greater toxicity⁴. To distinguish between organolead species from automotive sources and from soil minerals added during the processing or storage of grapes, the use of species-selective and sensitive analytical techniques is essential.

We have analysed 19 vintages of a renowned French wine, *Château-neuf-du-Pape*, made of grapes collected in precisely the same area for 40 years. We used a custom-designed procedure to discriminate between Pb²⁺ and particular organolead (nonpolar and ionic) species⁵. The vineyard of origin is at the junction of two heavily-used French auto-routes (A7 and A9), and thus is subject to intensive automotive pollution. Variations of the two most abundant compounds, trimethyllead and triethyllead, the direct degradation products of tetramethyllead and tetraethyllead which are used as antiknock additives in petrol, are shown in the figure.

The concentration of organolead in the wines undergoes considerable variation with vintage. The values measured are 10–100 times higher than those in drinking water⁶; at the maximum they reach levels up to 0.5 µg l⁻¹, which is relevant to health concerns. The pattern of trimethyllead variation follows the consumption of leaded gasoline in western Europe (ref. 7 and R. Fiat, personal communication). No methyllead was found in the 1950 vintage, since this compound was first introduced only in 1960 (ref. 8); apparently no natural biomethylation of inorganic lead occurs in wine.

The introduction of regulatory measures to reduce the lead level in petrol at the beginning of the 1980s is reflected by a sharp decrease in the contamination of wine. On the other hand, triethyllead is



Changes in the concentration of a, trimethyllead; and b, triethyllead as a function of vintage.

already present in the oldest vintage investigated (1950). Its concentration triples in 1962, drops sharply in the mid-1960s as a consequence of a sudden increase in the fraction of methyllead used and then slowly decreases throughout the 1980s owing to the decrease of leaded petrol consumption.

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Global change detection

SIR — Considerable effort is being focused on the detection of an anthropogenic climatic change signal in short (10–15-year) satellite-derived datasets, such as lower tropospheric temperatures from the microwave sounding units (MSUs)¹ or sea-surface temperatures (SSTs) derived from the advanced very-high-resolution radiometers or the current and future along-track scanning radiometers (ATSRs).

J.R.C. and R.T.McN. reported an apparent warming trend in the MSU temperature record, after correcting for the effects of El Niño and volcanic aerosols². To place this result in perspective, we need to evaluate the probability that it occurred by chance. Applying ordinary least squares (o.l.s.) regression to the corrected MSU record from January 1979 to March 1994 (183 months), we find a warming trend of 0.069 °C per decade (the discrepancy with ref. 2 is due to the inclusion of additional data and an adjustment required for the NOAA-11 instrument). If we assume that the decadal trend measured by the MSUs represents the true trend to within ±0.03 °C per decade in 98% of cases¹, o.l.s. regression indicates that this trend is significantly different from zero at the 99% level.

Significance estimates based on o.l.s. regression assume, however, that monthly global temperature anomalies, T' , after the effects of El Niño, volcanoes and any trend have been removed, are not self-correlated in time. This is unlikely, as T' is a naturally integrated quantity. Suppose we can identify a timescale, δt , beyond which heating anomalies, Q' , are not self-correlated. To first order, short-term fluctuations in T' may then be represented by the AR(1) model³:

$$T'(t + \delta t) = T'(t) e^{-\delta t / \tau} + \frac{Q'(t) \delta t}{c}$$

where τ is the characteristic relaxation timescale for temperature anomalies, and c is the heat capacity of the system. Provided $\tau \neq 0$, T' will be self-correlated. Thus, although the real climate clearly involves more than one heat capacity and relaxation timescale, the AR(1) model is a natural approximation which turns out to be a surprisingly good description of the behaviour of the global mean temperature on less than 40-year timescales⁴.

The standard Durbin–Watson test⁵ indicates that the o.l.s. residuals from the corrected MSU record are indeed self-correlated at a very high confidence level. We therefore have both theoretical and empirical reasons to distrust o.l.s. results. We can take this self-correlation into

account by applying estimated generalized least-squares (e.g.l.s.) regression^{3,6} to the corrected MSU record with an AR(1) model for the residuals. This gives a warming rate and standard error of 0.063 ± 0.047 °C per decade: the null hypothesis of no warming cannot be excluded even at the 85% level. With this revised estimate of the error in the trend from the MSU data, a warming rate of 0.15 °C per decade (the lowest rate pro-

range $0-0.5$ °C per decade, to simulate the possible effect of greenhouse-gas-induced warming over the coming decades (predictions range over $0.15-0.4$ °C per decade⁷, but these do not take into account the possible cooling effects of sulphate aerosols and century-timescale climate fluctuations). We then tested for a warming trend in short segments of these artificial series, using e.g.l.s. regression with an AR(1) model for the residuals (see

figure). If we are confident that the long-term drift in our observing system is zero, a 15-year dataset appears to give us an 80–95% chance of detecting a warming trend if the true trend is 0.25 °C per decade (point *a* in the figure).

If, however, we can only guarantee that the observing system has drifted by ≤ 0.15 °C per decade, we might then require a trend > 0.15 °C per decade before claiming unambiguous detection. The probability of detection in a 15 year-data set then drops to $\sim 50\%$ (point *b* in the figure). This highlights the importance of strict control of observing system drift, which can be achieved only

continuous cross-instrument validation. Removing the El Niño signal (by regression on a lagged, low-pass filtered Southern Oscillation index¹⁰) may reduce these detection times by 1–2 years. The use of seasonal in place of monthly SSTs increases typical detection times by about 2 years, indicating that the noise-reducing effect of taking a 3-month average is more than compensated for by the increased sampling interval.

Thus, 10–15 years represents the shortest length of dataset in which we should expect to be able to detect a warming trend of 0.25 °C per decade. Our failure to detect a significant trend in the MSU dataset may allow us to exclude warming rates at the upper end of the current uncertainty range, but it should not be regarded as conclusive

evidence that no warming is taking place, particularly if there is still any doubt about the stability of the observing system.

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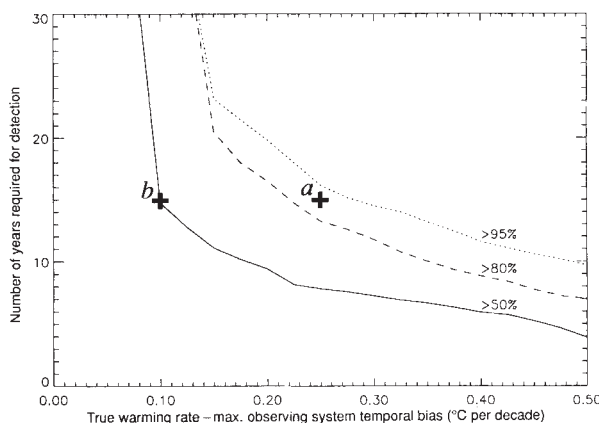
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Estimated probability of detecting a warming trend in global monthly mean SST (e.g.l.s. regression with AR(1) model; see text). For all true-trend (minus possible observing system drift) and segment-length combinations above the solid/dashed/dotted line there is a $> 50/80/95\%$ chance of detection at the 97.5% confidence level ($\sim 2\sigma$ for a one-tailed *t*-test).

posed by ref. 7 on the basis of model predictions) also lies just within the conventional $\pm 2\sigma$ range.

Given this failure to detect a significant warming trend in the corrected MSU data, what is the probability of detecting a warming trend of the magnitude predicted by climate models in any 10–15-year dataset, given the level of natural climate variability? As a proxy for natural variability on the relevant timescales, we use monthly global mean SSTs from historical ship observations⁸, with the residual annual cycle and all variability on timescales greater than 60 years removed using a nonlinear-trend reconstruction algorithm⁹. Varying this cut-off time over 40–80 years has little impact on the results. Global SST was chosen because the level of ‘noise’ is likely to be no higher in this parameter than in any other¹. We use these detrended SSTs to represent the interannual to interdecadal variability which will be present in any global temperature series, however observed. To minimize the short-term effects of sampling and observation errors, we use only post-1900 data (spurious long-term drifts will have been removed by the detrending). Some such variability will remain, so our estimated detection times will be slightly conservative, but only for a completely noise-free observing system.

We added linear trends to the 1901–90 detrended SSTs, with gradients in the

Intrinsic source of stomach NO

SIR — Benjamin *et al.* have discussed the interesting possibility that swallowed salivary and food-derived nitrate may give rise to nitric oxide (NO) generated in the stomach, where it might be a defence mechanism against swallowed pathogenic microorganisms¹. As well as this effect, NO has previously been reported² to protect the gastric mucosa from acid-induced lesions.

But NO in the stomach may not be delivered only by extrinsic sources but may also be generated by an intrinsic epithelial cell type of the gastric surface epithelium long known under the names brush cell, tufted cell or caveolated cell³. This particular cell type is scattered throughout the surface epithelium in the stomach of humans and probably all other mammals⁴. In the rat, brush cells are particularly abundant in the cardiac region of the gastric mucosa⁵.

Our belief that brush cells provide an intrinsic source of NO is based on the following observations of brush cells in the rat stomach:

(1) All brush cells display strong immunoreactivity with an antibody specific for the brain type of NO synthase⁶ (*a* in the figure). Immunostaining is abolished by absorption of the antibody with purified NO synthase from rat cerebellum.

(2) In immunoblots of electrophoretically separated proteins of the cardiac epithelium, the NO synthase antibody detected a M_r 160,000 polypeptide band displaying the same electrophoretic mobility as neuronal NO synthase from rat brain (*d* in the figure). In the oxyntic mucosa, which contains only relatively few brush cells, NO synthase is not

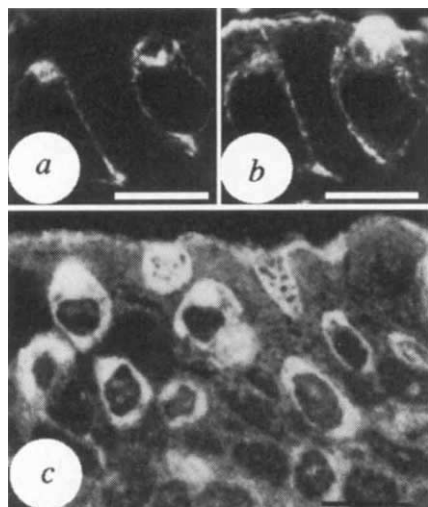
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detectable by immunoblotting.

(3) Brush cells display high enzyme histochemical activity for NADPH diaphorase. This NADPH-oxidizing activity is probably provided by a cytochrome P450 related domain of the NO synthase sequence⁷. NADPH is essential for NO generation.

(4) In brush cells, NADPH appears to be mainly delivered by glucose-6-phosphate dehydrogenase, which we find to be particularly abundant in brush cells by both immunostaining and enzyme histochemistry (c in the figure).

We conclude that brush cells in the stomach surface epithelium provide an



Enzymes involved in NO generation in brush cells of gastric epithelium (cardiac region) of the rat. *a, b*, Semithin tissue sections of unfixed freeze-dried and plastic-embedded tissue stained by immunofluorescence with antibody against synthase NO (*a*) and villin (*b*), a marker for brush cells⁵ (scale bars, 10 μ m). *c*, Demonstration of glucose-6-phosphate dehydrogenase (G6PD) by immunofluorescence in brush cells (grazing section) by an antibody against yeast G6PD that crossreacts with mammalian G6PD (ref. 8) (scale bar, 10 μ m). *d*, Immunoblot analysis of electrophoretically separated proteins of the cardiac epithelium using antibodies against NO synthase (lane 1), villin (2) and G6PD (3). NO synthase and G6PD in the stomach show the same apparent relative molecular mass (electrophoretic mobility) as NO synthase of the rat cerebellum (4) and purified yeast G6PD (5), respectively.

intrinsic source of NO in the gastric lumen where NO, among other functions, may help to protect against gut pathogens, as suggested by Benjamin *et al.*¹. As gastric brush cells display ultrastructural features shared by taste receptor cells but do not show any association with nerve fibres, it is possible that brush cells can sense the chemical milieu of the stomach contents and respond with the production of NO as messenger molecule and/or microbicidal agent.

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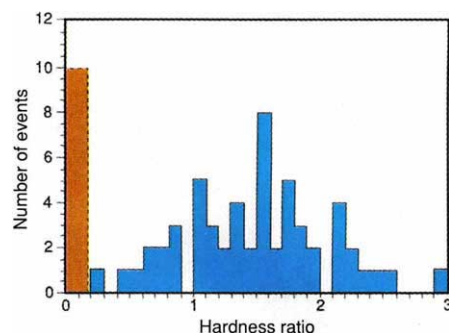
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Gamma-ray bursts still a mystery

SIR — Several times in the past few months I have come across some confusion about whether soft gamma repeaters (SGRs) are some kind of repeating gamma-ray bursts (see, for example, ref. 1). On behalf of my colleagues in the BATSE team, I would like to clarify the matter.

The first two SGRs were discovered² in 1979 with instruments designed to detect gamma-ray bursts (GRBs); hence the origin of the confusion. Originally, they were classified as repeating, short, soft (low-energy) GRBs, for lack of more evidence. Their unusual characteristics were noted² but the subject remained closed until 1986, when it became clear with the discovery of the third source^{3–5} that SGRs might be related to galactic populations⁵. Conversely, the BATSE results⁶ strongly indicate that GRBs are extragalactic objects, and therefore that they are an altogether different kind of phenomenon.

When the Compton Gamma Ray Observatory was launched, the BATSE team had, as one of its main objectives, the detection of new SGR sources. Because of its high sensitivity, BATSE had a good chance of detecting SGR emissions of similar or weaker intensity than those detected previously. In our first three years of operation we detected^{7,8} the reactivation of two of the old sources, but no new ones. This indicates that these objects are very rare in our Galaxy. The



Frequency distribution of the hardness ratios of SGRs (orange) and short GRBs (blue).

galactic (neutron star) nature of the objects has been argued for in the past^{5,9}; the recent articles in *Nature*^{7,10,11} in which SGR 1806–20 has been identified with a plerionic supernova remnant in our Galaxy confirm this hypothesis.

Can we distinguish between SGRs and short GRBs? Or, in other words, how many SGR emissions have been misidentified in our database? To answer this question, I present here a histogram of the hardness ratios of the SGR emissions detected with BATSE, and of those of the short (< 2 seconds) GRBs detected during the first year of our operation. I define the hardness ratio as the ratio of the counts detected between 100 and 300 keV to those between 50 and 100 keV. The figure shows that there is a clear separation between the two types of events. We estimate that at most one SGR emission could have been mistaken for a GRB and vice versa. We are therefore confident that we can separate these two different types of transient phenomena based on their distinct spectral characteristics alone.

Finally, I would like to stress again the differences between SGRs and GRBs. The former are sources of randomly repeating, low-energy, very short emission, currently identified^{7,10,11} with neutron stars in our Galaxy. The latter cover a wide range of durations and energies; they have not yet unambiguously been shown to repeat; and they are not compatible with the distribution of any known population of objects in our Galaxy. They remain one of the great unsolved mysteries in high-energy astrophysics.

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Power to the people

Ian Fells

Nuclear Renewal: Common Sense About Energy. By Richard Rhodes. Whittle Books/Viking (US): 1993. Pp. 127. \$17.50.

Nuclear Madness: What You Can Do. By Helen Caldicott. Norton: 1994. Pp. 240. \$22 (hbk); \$10.95 (pbk).

Prospects and Strategies for Nuclear Power: Global Boon or Dangerous Diversion? By P. Beck. Earthscan/Royal Institute of International Affairs: 1994. Pp. 118. £12.95 (pbk).

Power from Plants: The Global Implications of New Technologies for Electricity from Biomass. By Walt Patterson. Earthscan/Royal Institute of International Affairs: 1994. Pp. 102. £9.95 (pbk).

"NUCLEAR power is not dead. It runs France and will soon run Japan", writes Richard Rhodes in *Nuclear Renewal*. He could have gone on to say that it provides two-fifths of the electricity for the European Union, more than any other fuel. And in the former Soviet republics, despite serious concern about the design and management of the RBMK Russian-designed reactors, the need for electric power is so great that the suspect reactors continue to operate. All in all, about 430 power reactors provide a fifth of the world's electricity.

The United States operates 110 commercial reactors, more than any other country, but its nuclear industry has been at an impasse since 1978, bedevilled by overspending, complacency, carelessness and inefficiency. "The failure of the US nuclear power programme", announced *Forbes* magazine in 1985, "ranks as the largest managerial disaster in business history." But there is more to it than that. When Enrico Fermi first generated half a watt of power from his newly critical atomic pile in a squash court in Chicago on 2 December 1942, the achievement heralded the advent not only of civil nuclear power but also — and more immediately — of the plutonium bomb and the nuclear submarine. Indeed, the compact, pressurized water reactor (PWR) unit of the submarine was scaled up to give the 1,300-MW PWRs of today; unsurprisingly, the Soviet Union went in the same direction.

Military nuclear power units were the first prototypes for the civil programme, despite the fact that the design was less than ideal for a civil reactor; a high degree of engineering safety must be included in a civil PWR, because when things go wrong, they go wrong quickly — witness the accident at Three Mile Island in the United States in 1979. The advanced gas-cooled reactor pioneered in the United Kingdom has an accident-time constant of 14 hours compared with 2 minutes for a PWR, but successful, aggressive US salesmanship has ensured that the world has bought PWRs, including France and now the United Kingdom.

It is ironic that the United States has not

ordered a nuclear station since 1978. Some of the reasons, apart from management failure, are given in Helen Caldicott's tract *Nuclear Madness*, an updated version of her original *cri du cœur* about the dangers of nuclear power, first published, as it happens, in 1978. She writes as a doctor, mother and world citizen and "wants to practice the ultimate form of preventive medicine by ridding the earth of these [nuclear] technologies that propagate disease, suffering and death". Her book is a sustained polemic against all things nuclear. She describes a series of Nazi-like radiation experiments apparently carried out in the United States on retarded boys, pregnant women, prisoners and cancer patients and described in recently released papers from the US Department of Energy. Some of her evidence is at variance with official reports. Referring to the Three Mile Island accident, she complains that no epidemiological studies have been conducted on the irradiated population; a feature of that particular accident, however, was the minuscule release of radiation. She comments on a Chernobyl-like accident where "thousands of people will die from immediate radiation exposure" — not a feature of the Chernobyl disaster. And she remorselessly piles horror on horror, complaining of a global nuclear "priesthood" still intent on building new reactors.

I have some sympathy when she says that "we can no longer afford to entrust our lives . . . to politicians, bureaucrats, 'experts', or scientific specialists because all too often their objectivity is compromised". But she damages her own case by providing an account that is anything but objective. As far as she is concerned, the nuclear industry and its supporters are evil. In fact, they are only engineers and administrators trying to do a job; bumbling, inefficient, complacent they may be, but they are not the spawn of the devil bent on destroying the world.

Both *Nuclear Renewal* and *Nuclear Madness* are strongly confrontational. Irritating though this may be, the strength of the authors' feelings carries the reader along. *Prospects and Strategies for Nuclear Power* by Peter Beck is an altogether

cooler look at the pros and cons of nuclear power. The author takes the trouble to explain first the history and then the technology of nuclear power generation and the nuclear fuel cycle, knowledge of which is central to any real understanding of the problem. He concludes gloomily that the most worrying lesson to be learned from the contemporary scene is that the argument between the pro- and antinuclear lobbies is as fierce and unresolved as ever. But being a pragmatist and also the founding father of 'scenario-planning techniques', he examines three cases: the prospects for "phase-out" of nuclear power, continuation of the present situation, and worldwide expansion. This is done against a backdrop of an insatiable desire for more electricity, particularly by the developing world, with demand doubling by 2020 and trebling by 2050. (The author warns however that there could easily be a 30–40 per cent margin of error for 2050.) The implications for electricity generation will depend greatly on whether concern about the greenhouse effect increases or decreases early next century; particularly vulnerable is the future of coal, which today has the lion's share of electricity generation at 42 per cent, but could sink to 23 per cent by 2050. The long-term future of oil lies in transport, although the prospect of a doubling or even trebling of the 600 million cars on our roads by 2020 is horrifying. Gas is the joker in the pack, rising from 12 per cent today to 20 per cent. Hydroelectric power will remain steady at 15 per cent, but the new renewable sources, biomass, tides, waves, wind and so on could rise to contribute between 10 and 35 per cent. This leaves nuclear power bumping along at between 10 and 15 per cent, possibly rising as high as 25 per cent by 2050 or being phased out.

The author concludes: "the world does need the flexibility for nuclear energy to become a major energy resource" but "the most important condition to be met is to achieve full acceptability by the public which in turn depends on ensuring that expansion does not increase the risk of proliferation and nuclear terrorism, that nuclear waste can be safely and acceptably disposed of and that nuclear facilities everywhere are sufficiently safe".

Beck's analysis is predicated on new renewable sources providing between 10 and 15 per cent of the electricity supply. In *Power from Plants*, a long-time antinuclear campaigner, Walt Patterson, focuses his attention on the future of biomass as a fuel. There is nothing new about wood as a fuel; even today it provides about 13 per cent of the world's energy, including non-commercial energy; and in some extremely poor countries, it provides as much as half their total energy. But firewood is running out. On the other hand, crops, including wood, grown specifically for fuel

and particularly on 'set-aside' land no longer needed for food, could be the most important renewable energy source for the next quarter of a century, according to the US Department of Energy: 25 gigawatts of biomass electricity could be in operation in the United States alone by 2010, as opposed to 6.5 gigawatts today. Biomass has the enormous advantage of being 'greenhouse neutral' and will be especially important in tropical and subtropical regions where demand for electricity is growing fastest. The effective use of biomass for electricity generation is a high-technology option, with gasification, combined-cycle generation and combined heat and power systems, at local and national level, all playing a part. Agriculture has not yet been optimized for biomass production, with the photosynthetic process disappointingly inefficient at around 1 per cent (5 per cent for some crops), and there is a need for more demonstration gasification plants. But support for biomass production is strengthening and the next 25 years should see — and indeed will need to see — real progress in the technology.

The futures of nuclear and renewable energy seem inextricably entwined. Fossil fuels will eventually run out and have to be replaced if the goal of sustainable development is to be achieved. More urgently, the prospect of increasing atmospheric pollution leading to irreversible and unpredictable climate change is a spectre beginning to haunt us. Some statesman-like political decisions now would help to safeguard our future. □

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Pet themes

Michael Rowan-Robinson

Cosmic Enigmas. By Joseph Silk. *American Institute of Physics Press: 1994.* Pp. 214. \$29.95, £20. UK distributors, *Oxford University Press.*

THE dominant figures of postwar astrophysical cosmology have been the late Yakov Ze'ldovich, of Moscow, and Jim Peebles, of Princeton. But running a close third in influence has been Joseph Silk. Born in London and educated at Cambridge, he then emigrated to the United States where, after studying for his doctorate at Harvard, he ended up at the University of California at Berkeley. There he is head of the theory group in the department of astronomy and in the Center for Particle Astrophysics. He is now honoured as the tenth writer in the American Institute of Physics's not very correctly named series "Masters of Modern Physics".

There can be no doubt that, as a guru of modern cosmology, Silk merits this accolade. He is also one of that small and worthy band of scientists who write about science for a wider audience. This collection contains some of his writings in this vein over the past 15 years or so, many of them from *Nature's* News and Views section. All the great themes of contemporary cosmology are here: the Big Bang, inflation, primordial nucleosynthesis, galaxy formation, dark matter, large-scale structure. Generally the longer pieces are more satisfying than the short ones. And although the historian of science will enjoy tracing the changing fashions of cosmological theory over the years, the re-

cent articles are to my mind much more interesting than the older ones. It might have been better to arrange the articles in chronological order so that the evolution of cosmological thinking could be clearly seen rather than to group them by topic, which results in some bewildering jumps in time and state of knowledge.

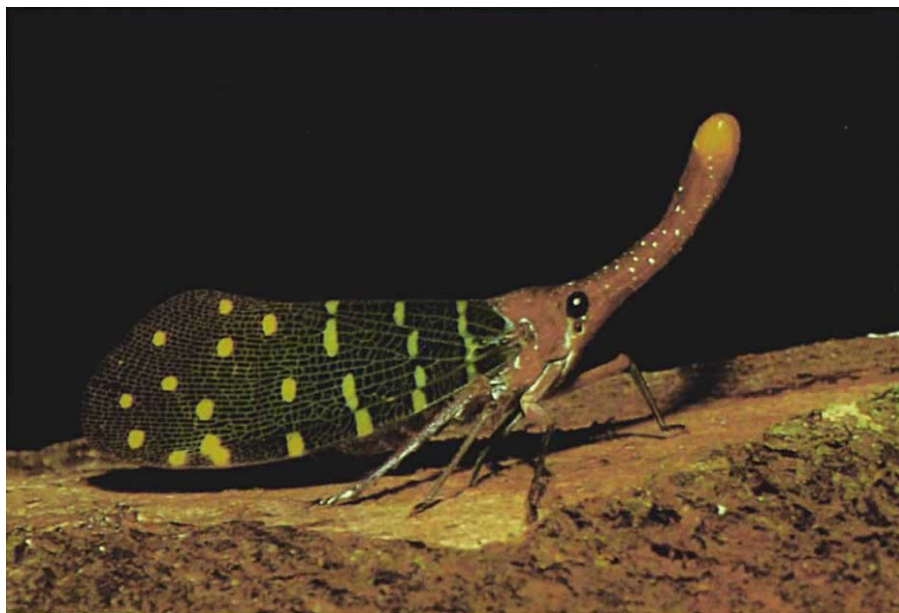
One of the best pieces, "Anthropic Musings", appears near the beginning of the collection and begins:

Once upon a time there was a flea, who lived on the back of a dog called Bonza. The flea thrived, and grew as big as a pea, for Bonza's fur was the perfect environment, neither too hot nor too cold, too dry or too moist, too dark or too light. Then one day the dog's mistress purchased a flea collar for her pet. From that day on, the world was a very harsh place for the flea, who no longer had a moment's peace. Sadly, the flea was forced to jump, and landed by a lamp-post, where it soon froze to death.

The moral of this story is that although a canine universe may be eminently well suited for the existence of fleas, fleas are not inevitable. Some dogs have them, some do not. It is equally presumptuous to believe that man's existence is inevitable, as advocated by proponents of what has become known as the strong anthropic principle.

Silk continues to pour scorn on the anthropic principle throughout this witty and pithy piece. One wishes that he would write more on these philosophical themes and issues, and in this satirical, almost Primo Levi-esque vein. Perhaps over the next 15 years he will do so. Meanwhile, this collection is essential reading for the cosmological enthusiast. □

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THIS green lantern bug (*Pyrops sultan*) makes an appearance in *Belalong: A Tropical Rainforest* by the Earl of Cranbrook and David S. Edwards. The richly illustrated book is the culmination of a 14-month exploration (1991–92) of pristine forest in the heart of South East Asia, on the northwest coast of Borneo. The study, a joint enterprise of the University of Brunei Darussalam in Borneo and the Royal Geographical Society of London, focuses on the forest's biodiversity and ecology. The authors have interwoven descriptions of the research with brief introductions to the climate and geology of the region as well as short explanations of the background science. The final two chapters address the long-term sustainability of this natural resource. Co-published by the Royal Geographical Society and Sun Tree Publishing Ltd (205 Henderson Road, 03-01 Henderson Industrial Park, Singapore 0351). £18 (hbk), £14.50 (pbk).

Reality rules

Andrew Pomiankowski

Games of Life: Explorations in Ecology, Evolution and Behaviour. By Karl Sigmund. Oxford University Press: 1993. Pp. 244. £49.95 (hbk); £17.95 (pbk).

GAMES are a way of thinking. They lie at the foundation of much theoretical work, especially recent developments in evolutionary biology. Insight has come from asking abstract and — at first sight — silly questions. What if inheritance was blending? How do populations evolve with three sexes? What is the fate of a gene that recognizes copies of itself in others by the presence of green beards? Thought experiments, by their distance from reality, allow general principles to be sought beyond the tangle of details. Playful investigation is not intended to deny reality but to help us come to grips with it. By creating a comprehensible half-way reality we may end up with a better understanding of the real thing.

The best introduction to this way of evolutionary thinking remains Richard Dawkins' *The Selfish Gene*. On the face of it *Games of Life* is an updated and advanced *Selfish Gene*. Although Sigmund is a mathematical biologist, he has made life easy for his readers by removing all mathematical technicalities. Simulations, diagrams and metaphors have replaced a glut of Greek symbols. At times the rewriting goes a bit too far and simple mathematics would have been easier to understand than the elaborate wordplay. But the result is a book accessible to all readers, whatever their level of numeracy.

There are excellent chapters on the foundations of Mendelian genetics, games of chance, John Horton Conway's computer game Life and the evolution of cooperation. Sigmund's aim is to promote "mathematical imagination". By this he means a willingness to think in abstract terms, using games and playing as a basis for thought experiments to start investigating problems such as the age of our youngest common gene or the advantage of sexual recombination. The approach makes these difficult questions seem easy and fun. He sells mathematics not as formulae, calculation and precision but as a way of thinking about the possible as well as the actual.

At times Sigmund gets a little carried away with his metaphors. In describing *Drosophila* segregation distortion, he asks readers to imagine two men (chromosomes) in a balloon (primordial sperm cell) who have to cross a mountain before landing, separating (meiosis) and then rushing off (sperm) to fertilize an egg. A primordial form of meiotic drive occurs if one man parachutes out of the balloon

before landing, thereby reaching the egg first. Parachutists spread even if they risk breaking their necks. But more sophisticated two-locus meiotic drive can now evolve. Non-jumpers who burn all parachutes will get rid of their parachuting companions who jump to their death. I'm not sure this makes meiotic drive completely transparent.

The content of *Games of Life* does not always live up to the promise of its style. For instance, the chapter on "Evolution and Sex" covers a lot of ground — mate choice, sex ratios, the reason why there are two sexes and the evolution of sex — but these topics have been well covered many times before and their appearance here has a slight textbook feel. Sigmund's games-eye view does not really constitute a novel perspective or lead to any new insights. Nevertheless, *Games of Life* is an excellent introduction to what theoretical biologists get up to in trying to understand evolutionary and ecological ideas. □

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Minds in the making

John C. Burnham

Inventing the Feeble Mind: A History of Mental Retardation in the United States. By James W. Trent Jr. University of California Press: 1994. Pp. 356. \$30.

In the nineteenth and twentieth centuries, Americans often set the standard for ways of treating — and institutionalizing — people labelled as mentally deviant, including those with mental retardation and developmental disabilities. Ten years ago, Peter Tyor and Leland Bell published a scholarly historical account, *Caring for the Retarded in America* (Greenwood, 1984), which is still useful for the basic narrative.

James W. Trent has now supplemented and to some extent reinterpreted their account. In contrast to Tyor and Bell's psychiatric orientation, he focuses on education and social work and is interested in how certain Americans became labelled with a disability and how the consequences of labelling changed over time.

Trent uses many primary sources, including two extensive archival collections, and he has also searched out secondary works. Written at a time of great change in historical scholarship, however, his account remains transitional.

The book falls into two parts. The longer part covers the period from the 1840s to the 1930s. Trent portrays the

superintendents of institutions for the mentally defective as motivated to achieve 'control', that is, to deprive their charges of liberty and to enlarge and protect their institutions and personal professional perks. This 'presentist' stance, combined with romantic individualism emphasizing elements of normality in retarded people, follows a pattern set in the 1970s by David Rothman's depictions of other kinds of institutionalization. The second part of the book, covering the period since the Second World War, is more sophisticated and explores the ironies of deinstitutionalization.

One of the main changes in which Trent is caught is the rapid retreat of social constructionism. Particularly in the history of science, of which his book is, broadly speaking, a part, advocates of the idea that scientific concepts are merely social artefacts are now recanting and admitting that scientists and doctors must incorporate nature into their attempts to understand the world. Indeed, right to the end of the first part of the book, after Trent has shown clearly how hereditary and environmental theories have alternated over generations, one expects him to conclude that mental retardation is in fact not real but simply a label imposed on victims of circumstance — that the new hereditarianism, popular in mainstream psychiatry, is an unpleasant (and 'conservative') social artefact. Yet in the last part, in which he is suddenly evenhanded, Trent leaves no doubt that mental retardation is a distressing fact of nature with which families, humanitarians and social policymakers must contend.

Both parts of the book are closely argued, although, curiously, along the lines of historical analysis rather than sociological theory. Indeed, the author uses concepts such as deviance, institutionalization and labelling without any obvious interest in the underlying theory. His central concept, control, remains undefined and largely rhetorical, so that the analysis, particularly in the first part, is incomplete. ('Control' does not appear in the index, which consists mostly of proper names.)

A constant feature in the history of mental retardation — apart from labelling — is underfunding. In the 1840s, some retarded children moved out of traditional family and almshouse settings and into training schools. By the late nineteenth century, schools had broadened their admission criteria and become custodial institutions. To attract state funding, they admitted adult residents and those with multiple disabilities. To have workers, they admitted the merely backward, who could work with supervision. Hereditarian beliefs provided the justification for the custodial movement (although Trent does not fully understand the details of degeneracy and Lamarckianism). As

one pioneer superintendent mourned in 1909: "Society only desires to get rid of [the mentally retarded] and be protected from them [whereas] the older ideas were to uplift them by every means that could be used. Now when thus congregated in Drovers like cattle it is about as much as we can accomplish to keep them comfortable and fed and clothed."

The 'feeble-minded' thus became 'mental defectives', and with a new implication: that their restraints on behaviour as well as their mental processes were blighted. They were the 'defective delinquents' who threatened social stability and prosperity. Because mental deficiency could be viewed as a simple Mendelian trait, the complementary strategies of segregation and eugenic sterilization were introduced. US communities also instituted special education classes and parole as it became clear that there were too many defectives for the institutionalization of all those unable to 'adapt'.

After the 1930s, institutions became discredited following underfunding and scandals, and new technologies (especial-

ly psychoactive drugs) began to lead to deinstitutionalization. Moreover, new advocacy groups, especially those involving family members, and university researchers began to replace superintendents as authorities on mental retardation. Reformers urged that rather than institutionalizing the retarded, normal community institutions should adapt to them ('mainstreaming'). Trent concludes that retarded people are not vegetables: many "hold full-time jobs, have families, and pay taxes — and wreck cars, have extramarital affairs, and get audited by the IRS [Internal Revenue Service]."

It is striking that, generations ago, the most severely mentally retarded were unable to cope with life in a producer culture, but in a consumer culture they have come to be consumers with the best of us — another redefinition of their — and our — essential humanity. □

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Dinosaur growth industry

Angela Milner

Dinosaur Eggs and Babies. Edited by Kenneth Carpenter, Karl F. Hirsch and John R. Horner. *Cambridge University Press: 1994. Pp. 372. £55, \$79.95.*

Do not be misled by the title, or by the cover illustration of a cute, gangling, down-clad titanosaurid hatchling. This is not a book aimed at the popular market but a collection of papers on all aspects of dinosaur reproduction and development for the professional reader and advanced student.

John Horner's discoveries of dinosaur nesting sites in the late Cretaceous strata of Montana, United States, during the late 1970s was the stimulus for a whole new slant on dinosaur research. Much of what is understood about dinosaur behaviour, reproduction and physiology is based on the study of nests, eggs, the growth and development of embryonic and juvenile skeletons, and the palaeoecology and environment of nest sites. This long-awaited book sets out to review and summarize today's knowledge of one of the most exciting areas of dinosaur studies.

The book consists of 24 separately authored chapters on distribution and history, nests, eggs and 'dinosaur babies'. The first chapter, a list of the global distribution of eggs, nests, bones, teeth and footprints, in chronological order, is a reference work in its own right.

Much of the information published here has been previously only tantalizingly

hinted at in abstracts or referred to in passing as work in progress, and the lack of hard data has been the cause of some scepticism among vertebrate palaeontologists. A case in point is altricial (young relatively undeveloped when born hatched) and precocial (young at relatively advanced stage of development when born hatched) breeding patterns. New evidence for both patterns appears in papers in the 'babies' section, lending support to an idea suggested by Horner and David Weishampel in 1988 that altriciality, found so far only in large derived eusauropsids, may be a function of adult body size in that group.

Dinosaur growth rate is another controversial topic where debate has outstripped published data. In the book there is much discussion and general agreement, on the basis of histological and ontogenetic observations, that young dinosaurs grew rapidly. A novel approach by Weishampel and Horner addresses the importance of life-history syndromes and heterochrony in understanding dinosaur evolution and the authors attempt to analyse key shifts in the framework of a cladistic phylogeny while acknowledging that the database is currently sparse indeed. Descriptive contributions on embryonic and juvenile dinosaurs illustrate criteria by which such material can be recognized. This may sound obvious enough but, until recently, many workers failed to realize that smallness may indicate juvenility rather than a new taxon.

Study of dinosaur eggs and eggshell structure has evolved into a discipline of its own with a taxonomy of egg families, genera and species. Most of the recent studies have adopted a common system pioneered by the Chinese worker Z. K. Zhao in 1975. Several chapters demonstrate its value in analysing associations of egg types and in interpreting nesting-behaviour patterns. So links and correlations can be made between taxonomic groups and environments in which the eggs were laid, for example between the rich Upper Cretaceous egg horizons in the Gobi Desert, central China and France — even though the identity of the egg-layers is uncertain.

Eggshell studies also yield information about chemical content, pathology and physiology, including gaseous exchange, type of nest and position of eggs in it. Several chapters review these topics and two authors draw attention to the importance of changes that can alter shell structure during lithification of a sediment; recrystallization of eggshell calcium carbonate is common.

The bulk of evidence on dinosaur reproduction comes from Cretaceous strata, but rare records from the Triassic of Argentina and early Jurassic of South Africa suggest that nest construction may have been plesiomorphic (that is, a hereditary character common to all dinosaurs). Egg-laying patterns are reviewed and two basic types, clutched (nest) and linear, are defined, with many variations of each type; a summary shows the extent to which the different types can be identified with dinosaur groups.

This work is an essential addition to the dinosaur literature and is the only compact reference work covering reproductive biology. It succeeds admirably in summarizing the current state of knowledge while drawing attention to how much remains unknown about dinosaur life histories. *Tyrannosaurus rex*'s is still a complete mystery. □

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■ **Solnhofen: A Study in Mesozoic Palaeontology** by K. W. Barthelemy, N. H. M. Swinburne and S. Conway Morris has just been published in paperback by Cambridge University Press (£16.95, \$24.95). One of the most famous limestones in the geological record, Solnhofen in southern Germany contains an astonishing diversity of organisms buried 150 million years ago, many of them exquisitely preserved: highlights include jellyfish, crustaceans, squid, fish, flying reptiles and — perhaps most famously — *Archaeopteryx*. "An excellent general survey... of the considerable body of research by German sedimentologists and taphonomists during the last 25 years" (*Nature* **249**, 114; 1991).

Revealing the blueprint of photosynthesis

J. Barber & B. Andersson

Recent research has revealed the nature of the reactions that underlie the conversion of solar energy into chemical energy by photosynthesis. Armed with this knowledge, a blueprint for new technologies to exploit solar energy is emerging.

PLANTS and photosynthetic bacteria capture and efficiently use sunlight by employing molecular machinery embedded in their membrane systems. Today, photosynthesis captures more than a hundred times the food requirement of mankind and is the origin of fossil fuels. Because of the photosynthetic process, atmospheric carbon dioxide is recycled every 300 years and oxygen every 2,000 years.

Judged on recent spectacular successes in determining the structure of several membrane proteins, the photosynthetic apparatus may be the first complex biological system to have its structure, function and regulation described in rigorous physical-chemical terms at the atomic level. Such knowledge could provide a blueprint for new technologies for the production of energy.

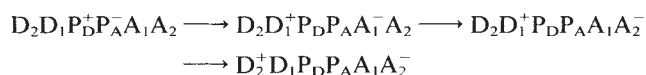
Light-driven charge transfer

The initial photosynthetic conversion of light energy to chemical energy takes place in pigment-protein complexes known as type-I and type-II reaction centres. The two types of reaction centre probably have a common evolutionary origin¹ as they apparently use a 'special pair' of chlorophyll molecules as the primary electron donor (P_D) and a chlorophyll or pheophytin as the primary electron acceptor (P_A), where the specific properties of these molecules (redox potentials and optical absorption) are fine tuned by their protein environments. When excited by light, P_D gives an electron rapidly to P_A , and a radical pair $P_D^+P_A^-$ is formed. The pigments are arranged in the reaction-centre proteins such that primary charge separation occurs across a lipid membrane with a low dielectric constant.



Early during evolution this transmembrane electron transfer process may have formed the basis of a primitive light-driven proton pump that used a mobile quinone molecule as the proton carrier (see Fig. 1a). A redox-active proton pump of this type would probably have incorporated additional electron transport systems leading to oxidation of substrate and reduction of carbon dioxide (Fig. 1b). Such an adaptation would not be possible for a light-driven proton pump of the bacteriorhodopsin type found in halophilic bacteria where the mechanism of proton translocation involves pH-induced conformational rather than redox changes^{2,3} (Fig. 1c).

The transfer of an electron from the excited primary donor (P_D^*) to P_A takes just a few picoseconds, avoiding de-excitation of P_D^* by other reactions and ensuring high quantum yields and efficiencies. This is achieved by the rapid transfer of the electron from P_A^- to secondary electron acceptors A_1 , A_2 and so on, and by passing an electron to P_D^+ from the secondary electron donors D_1 , D_2 and so on.



Today, type-I and type-II reaction centres are distributed between two distinct classes of anaerobic photosynthetic bacteria. Green sulphur photosynthetic bacteria and heliobacteria contain type-I reaction centres characterized by having low-

potential iron-sulphur centres as terminal electron acceptors. In contrast, type-II reaction centres use quinones as their terminal electron acceptors and are found typically in sulphur and non-sulphur purple photosynthetic bacteria and certain green bacteria, such as *Chloroflexus*. Both types of reaction centre occur in aerobic cyanobacteria, algae and plants where they are coupled together in series so as to oxidize water and create the low redox potential needed to reduce $NADP^+$. Reduced $NADP^+$, with ATP, is then used to convert carbon dioxide to organic molecules. The oxidizing potential required to split water is provided by a type-II reaction centre known as photosystem II (PSII). Correspondingly, the iron-sulphur-containing type-I reaction centre of oxygenic organisms, photosystem I (PSI), uses electrons made available by PSII to reduce $NADP^+$ (Fig. 2).

In addition to PSI and PSII there are two other membrane complexes involved in $NADP^+$ reduction and ATP formation. A cytochrome-containing complex, cytochrome b_6f , acts as a redox link between PSII and PSI (see Fig. 2), whereas the generation of ATP takes place at the 'coupling factor' complex, CF_0 - CF_1 . This complex uses the transmembrane proton gradient, generated by light-driven electron transfer from PSII to PSI, to convert ADP to ATP in line with Mitchell's chemiosmotic theory⁴. In the case of the thylakoid membrane of plants and algae, 60 different proteins are associated with the energy-converting systems. In eukaryotes, about half of these proteins are encoded within the chloroplast DNA, which has been sequenced in its entirety⁵. Curiously, only a dozen or so of the 60 known proteins are directly involved in the energy transduction process.

Type-II reaction centres

The elucidation of the structure of the reaction centre of the purple bacterium *Rhodospseudomonas viridis*^{5,6} revealed a 2-fold symmetrical relationship of its prosthetic groups and of the two protein subunits (L and M) that bind them. These two subunits possess five transmembrane helices each, and the precise distances and orientations of the components involved in the electron transfer events are now known to atomic resolution. The primary electron donor, P_D , is a special pair of two bacteriochlorophylls, $(BChl)_2$, placed on the periplasmic side of the reaction centre. Following the structure across the membrane, the next two cofactors are two bacteriochlorophylls ($BChl_L$ and $BChl_M$), then two bacteriopheophytins (BPh_L and BPh_M), followed by two quinones contained in the Q_A and Q_B sites near to the cytoplasmic surface. All the duplicated cofactors are related by a 2-fold axis passing through $(BChl)_2$ and a non-haem Fe^{2+} placed midway between Q_A and Q_B (see Fig. 3). Despite this symmetry, electron transfer from $(BChl)_2$ to Q_B through Q_A , occurs almost exclusively along the L-branch.

The $BChl_L$ molecule of the purple bacterial reaction centre is located between $(BChl)_2$ and BPh_L . It is not yet known whether this chlorophyll molecule serves as a conventional redox cofactor⁷, an alternative possibility being that it mediates electron transfer from $(BChl)_2$ to BPh_L without being directly involved as a redox-active species.

The properties of the bacterial reaction centre, particularly the preferred use of the L-branch for electron transfer, are not easily explained by current electron transfer theories⁸⁻¹⁰. More-

over, site-directed mutagenesis has so far failed to open up to the M-branch as an efficient electron transfer pathway, although certain mutations do affect the kinetics of primary charge separation mediated by the L-branch¹¹⁻¹⁵. On the basis of the theory of Marcus⁸, Moses *et al.*¹⁶ have used the properties of the bacterial reaction centre to conclude that the predominant factor controlling electron transfer rates in all proteins is the edge-to-edge distance of the donor and acceptor together with lesser, but vital, contributions from the values of Gibbs free energy (ΔG^0) and the associated reorganization energy of the reaction. Whether this concept is applicable under all conditions is unknown¹⁷.

The reaction centre structure is also serving as an invaluable model for studying proton, as well as electron, transfer in proteins because the Q_B quinone has to be protonated before it is detached from the L subunit¹⁸⁻²⁰.

The study of photosynthesis in plants has been aided by the appreciation that the plant PSII carries out electron transfer reactions similar to those occurring in the reaction centres of purple bacteria¹. Moreover, the two reaction centre subunits of PSII (D1 and D2) show homology with the L and M bacterial proteins^{21, 24} and form a heterodimeric reaction centre^{25, 26}. The primary electron donor of PSII, P680, is a powerful oxidant in its oxidized form²⁷; its potential is about +1.1 V as compared to about +0.4 V in bacterial systems. Indeed, P680⁺ is the most oxidizing species found in living organisms and is required for

extracting electrons from water. P680 is probably a dimer of chlorophyll *a* but the relative orientation of the chromophores and their degree of exciton interaction seems to be notably different to that of the primary donor (BChl)₂ in purple bacteria²⁸. The uniqueness of the redox properties of P680 poses the question as to what allows this highly oxidizing species to control the extraction of electrons from water rather than from nearby proteins.

In performing the chemistry of water oxidation, PSII requires the accumulation of four oxidizing equivalents that are necessary to abstract four reducing equivalents ($4e^-/4H^+$) from two water molecules to produce one molecule of dioxygen^{29, 30}. This accumulation of oxidizing equivalents involves a cluster of four manganese atoms which seem to be converted to higher valency states³¹ by four separate photochemical events involving P680 and a redox-active tyrosine residue at position 161 in the D1 protein³². The Mn cluster is intimately associated with the luminal surface of the D1/D2 proteins^{33, 34}. On the reducing side, PSII is similar to the anoxygenic purple bacterial system in having pheophytin and quinone molecules, Q_A and Q_B , as electron acceptors. Although the chemical species of these cofactors differ between the two systems, they possess essentially the same redox values and undergo electron transfer with similar rate constants. It is these similarities which characterize the reaction centres of PSII and purple bacteria as type II.

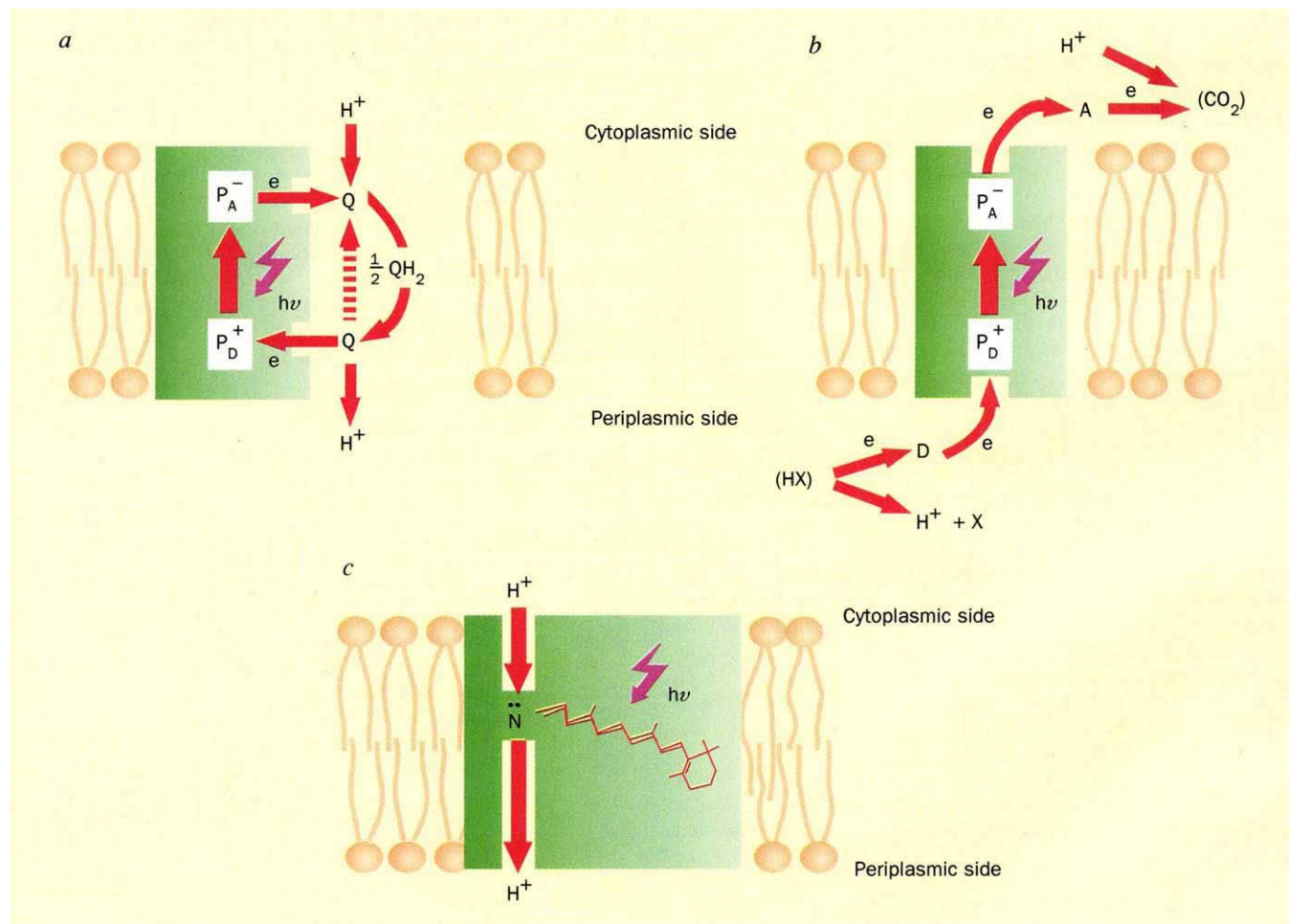


FIG. 1 Representation of a, a hypothetical redox-driven proton pump using a light-induced charge separation between a pigment electron donor (P_D) and a pigment electron acceptor (P_A) on opposite sides of a membrane and a quinone (Q) acting as a mobile hydrophobic electron/proton carrier. (This generalized model is the basis of a classical redox loop implicit in the Mitchell chemiosmotic hypothesis⁴.) b, A modified version of scheme a so as to use the redox potentials of the light-induced species P_D^+ and P_A^- to oxidize a suitable substrate (H_2X) and

reduce CO_2 , respectively (D and A are included to emphasize the involvement of a series of redox active intermediates in the overall electron transfer process. Note that schemes a and b are one electron/one photon events although in practice quinones are two electron/two proton carriers. c, A conformational transmembrane proton pump based on our present knowledge of the mode of action of bacteriorhodopsin which involves an acid/base induced *cis/trans* isomerization of bound retinal⁹.

Type-I reaction centres

The primary electron transfer events of the type-I reaction centre are, in many respects, similar to those of the type-II system. Again a special pair of chlorophylls are thought to act as the primary electron donor, although, unlike type-II reaction centres, the oxidizing potential generated, about +0.4 V, is similar in both oxygenic and anoxygenic organisms. The first acceptor is thought to be a monomeric chlorophyll and the subsequent electron transfer includes a quinone molecule. In contrast to Q_B of type-II reaction centres, this quinone in its reduced state, is not protonated and has a very low redox potential so that it can donate an electron to the terminal acceptor which is a 4Fe-4S centre. All these redox-active cofactors of the type-I system are bound to two similar or identical proteins that form a dimer thought to have a 2-fold symmetry similar to that found with type-II reaction centres. A striking difference between the protein dimers of type I and type II, however, is that the former binds a large number of light-harvesting chlorophylls. The low-potential 4Fe-4S centre ($E_m -0.7$ V) reduces NAD^+ or $NADP^+$ through other iron-sulphur centres which are incorporated in proteins associated with the outer surface of the reaction-centre dimer (see Fig. 2).

A preliminary X-ray diffraction analysis³⁵ has clearly revealed the positioning of the 4Fe-4S centres of the PSI reaction centre of the cyanobacterium, *Synechococcus* sp. The crystallized complex consists of several polypeptides and 16 of the 28 helices

detected probably belong to the reaction-centre dimer. Two chlorophylls have been identified as the primary electron donor P700 and the location of the chlorophyll and quinone acceptors tentatively assigned. As with type II, the PSI reaction centre appears to have a 2-fold symmetry with the axis passing through P700 and the 4Fe-4S centre.

Light-harvesting systems

Light-harvesting pigment proteins enable photosynthetic organisms to use the whole of the solar spectrum available and to grow at low light intensities. Understanding how chromophores are arranged in their protein environments is an important goal in the study of photosynthesis.

Kuhlbrandt *et al.*³⁶ has recently determined the structure of the light-harvesting chlorophyll *a/b*-protein complex of PSII (LHCII) at 3.4 Å resolution by electron crystallography. The LHCII binds about half of the total chlorophyll on Earth and is the most abundant membrane protein in chloroplasts. The monomeric form of the complex binds a minimum of 12 chlorophylls, and 2 carotenoids (xanthophylls), and previous electron diffraction studies have revealed the existence of three transmembrane helices with the two longest being tilted relative to the membrane normal³⁷. The new crystallographic data shows that these two helices are held together by ion pairs formed by charged residues in the hydrophobic core of the complex. The structural data suggest that these charged residues also serve as

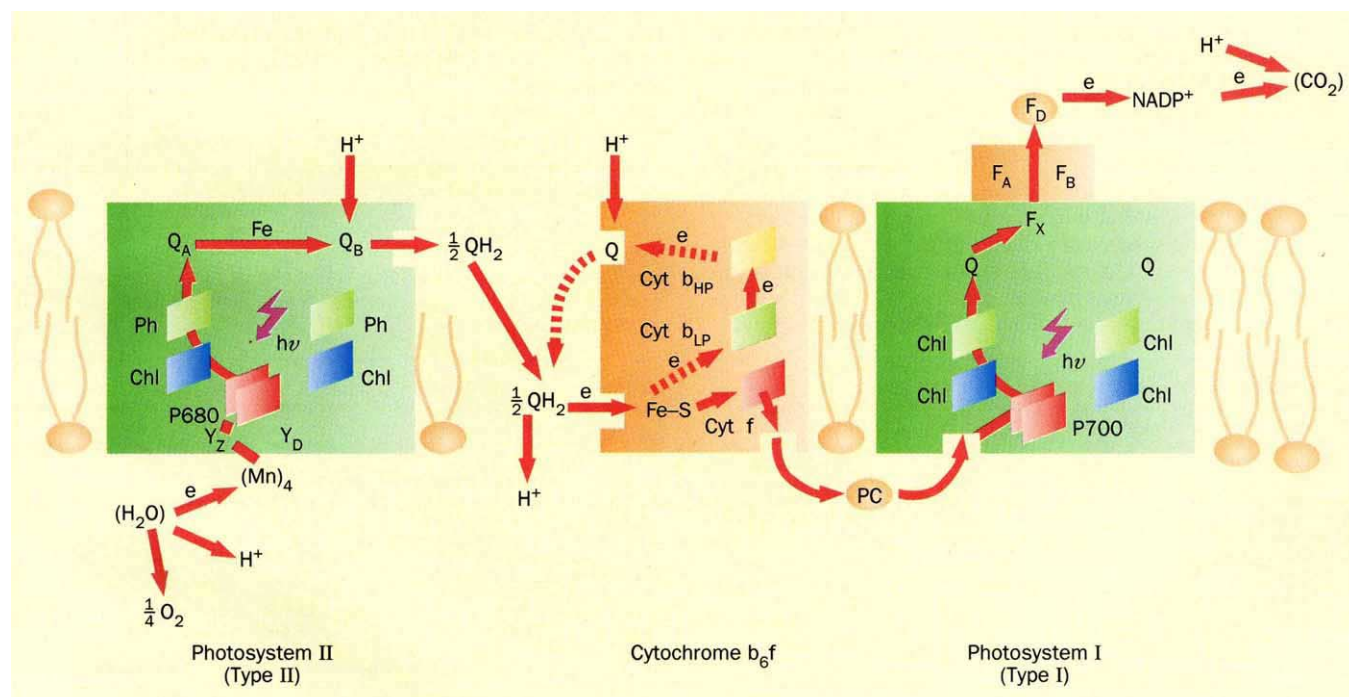


FIG. 2 A combination of the schemes a and b in Fig. 1 whereby a system is formed that uses the cooperation of two different photosystems to extract electrons and protons from the most abundant substrate, water, and make them available, to reduce carbon dioxide. The quinone reducing system is driven by a type II reaction centre called photosystem two (PSII), whereas the reaction centre responsible for oxidizing the reduced quinone and creating sufficient reducing potential to fix CO₂ is called photosystem one (PSI), being a typical type I reaction centre. Both types of reaction centre are depicted as having their cofactors arranged around a 2-fold symmetry axis as revealed from X-ray crystallography of reaction centres of purple photosynthetic bacteria^{5,6} and photosystem one³⁵. The latter structure is not resolved to atomic distance and, in any event, type I and type II reaction centres will have their own specific organizations according to their origin (oxygenic versus anoxygenic organisms). Chl, chlorophyll *a*; Ph, pheophytin (in the case of purple or green photosynthetic bacteria these cofactors are either bacteriochlorophyll or bacteriopheophytin). (Chl)₂, Some form of 'special' pair or interacting chlorophylls which act as the primary electron donor P₀

called P680 for PSII and P700 for PSI. Q, Quinone, plastoquinone in PSII and phyloquinone in PSI. Fe, Non-haem iron, F_xF_A, F_B are low potential iron sulphur centres and F_D is ferredoxin. (Mn)₄, The cluster of manganese atoms involved in the H₂O oxidation process; Y_Z and Y_D are redox-active tyrosines with the former being on the main route of electron transfer. Arrows show electron transfer paths. In oxygenic organisms, PSII and PSI are functionally connected by the cytochrome b₆/f complex which oxidizes the reduced quinone (QH₂, plastoquinol) and reduces a copper-containing extrinsic protein, plastocyanin (PC). Because quinones are normally 2 electron/2 proton acceptors, the intermediate cytochrome complex accepts two electrons, one reducing an iron-sulphur centre (Fe-S) and the other reducing a low potential *b*-type cytochrome (cyt_{b_L}). The reduced Fe-S centre donates an electron to PC through a bond cytochrome (cyt_f). The other electron participates in a quinone-dependent cycle involving high (Cyt b_H) and low (Cyt b_L) potential *b*-type cytochromes, which functions as a proton pump (Mitchell's Q cycle).

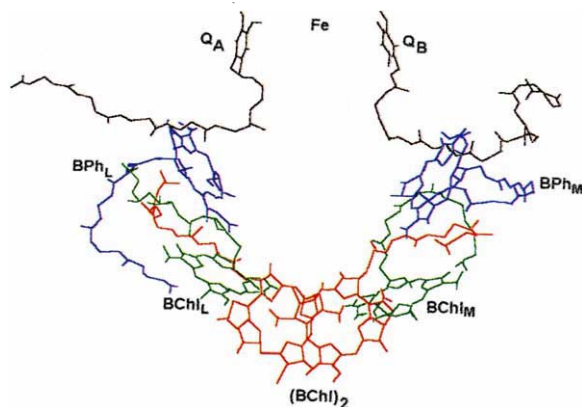


FIG. 3 Cofactor organization of the reaction centre from *Rhodospirillum rubrum*⁵¹. The symmetry axis is aligned vertically in the plane of the paper and passes through a bacteriochlorophyll dimer or special pair (BChl)₂ and a non-haem iron (Fe) the function of which is unknown. Electron transfer occurs from the excited (BChl)₂ through BChl_L monomer (possibly, see text) to BPh_L along the L-branch to Q_A and then to Q_B (BChl, bacteriochlorophyll; BPh, bacteriopheophytin; Q_A and Q_B are ubiquinone in this organism).

ligands for chlorophyll binding. The close proximity of chlorophylls *a* and *b* allows the rapid (1 ps) transfer of energy, and carotenoid groups are strategically placed to desensitize the chlorophyll triplet state and the resultant formation of highly reactive singlet oxygen, thereby performing a photoprotective role.

Some light-harvesting pigment proteins of prokaryotes are water soluble and their structures are known. The organization of the pigments within the protein is invariably ideal for energy transfer where the interpigment distance is short (10–15 Å), but not so short that wasteful quenching of excitations can occur.

Light-harvesting systems are able to respond to unpredictable changes in illumination. A 100-fold fluctuation in solar radiation can occur within seconds, often out of phase with the biochemical requirements of the organism. Excess light can lead to photo-

inactivation and destruction, particularly of PSII⁴¹, and mechanisms therefore exist to regulate light absorption⁴² and to repair damaged reaction centres^{43,44}. Interestingly, many of the regulatory mechanisms operate through changes in LHC-II organization and phosphorylation.

Artificial photosynthesis?

In terms of primary energy conversion, the secrets of the molecular electronics of photoreaction centres is now available to us at atomic resolution, and such data is becoming available for a number of different light-harvesting proteins. The challenge is now to devise an artificial system based on this knowledge. Recently, an artificial photochemical system has been constructed⁴⁵ that not only catalyses a photoinduced long-lived radical ion-pair state, as had been achieved previously⁴⁶, but one that possesses a recombination reaction able to generate a spin-polarized triplet state. Such a signal was discovered 20 years ago⁴⁷ and is characteristic of all photoreaction centres, but only recently has it been observed in a non-biological system. Another important advance has been the use of synthetic and genetically engineered proteins as the basis of self-assembly systems^{48–50}, allowing the insertion of pigments and cofactors into predetermined protein environments.

The emergence of innovative technologies based on the principles of the photosynthetic process will undoubtedly rely on developments in material science. For example, non-biological materials and electrochemical systems can be used as photoconverters, and one such system already developed⁵¹ uses dye-sensitized colloidal TiO₂ films that generate electricity with good efficiency and is constructed from low-cost materials. Initially, these technologies are likely to be limited to the construction of micro-optoelectronic devices for specialized use. But in the longer term a low-cost power generating system may be developed allowing us better to exploit solar energy. □

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ACKNOWLEDGEMENTS. We thank S. Zara for producing the diagrams in this article and colleagues who read the draft.

Detection of intergalactic ionized helium absorption in a high-redshift quasar

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Observations obtained with the recently refurbished Hubble Space Telescope reveal strong absorption arising from singly ionized helium along the line of sight to a high-redshift quasar. The strength of the absorption suggests that it may arise in a diffuse ionized intergalactic medium. The detection also confirms that substantial amounts of helium existed in the early Universe, as predicted by Big Bang nucleosynthesis theory.

BECAUSE the light emitted by high-redshift quasars traverses a significant fraction of the observable Universe, the absorption spectra of quasars provide unique and highly sensitive probes of gaseous foreground material encountered along the line of sight. The study of absorption lines in quasars has in recent years led to a dramatic increase in our knowledge of the presence and physical state of gaseous matter in the remote Universe.

If classified according to their major constituent, neutral hydrogen, two main types of intervening clouds give rise to discrete absorption line systems in quasars. The 'Lyman forest' clouds^{1,2} have neutral hydrogen column densities in the range $N_{\text{HI}} \approx 10^{13} - 10^{17} \text{ cm}^{-2}$. These ubiquitous systems give rise to a redshift-smearred 'forest' of lines seen on the short-wavelength side of emitted Ly- α in the spectra of all quasars and at all redshifts. The Lyman forest systems apparently possess little or no heavy elements, and are therefore generally believed to represent a population of intergalactic clouds of possibly primordial composition¹.

In contrast, the 'Lyman limit' systems³⁻⁶ nearly always exhibit matching lines due to heavy elements (C, N, O, Si and Fe) and are therefore associated with stellar-processed material in gaseous galaxy halos. The Lyman limit systems are more than an order of magnitude scarcer than the Lyman forest systems, but their column densities are considerably larger: $N_{\text{HI}} \approx 10^{17} - 10^{21} \text{ cm}^{-2}$. 'Lyman limit' systems are so called because they are opaque at wavelengths below the 912 Å photoionization threshold of neutral hydrogen, and therefore give rise to characteristic 'Lyman limit' absorption edges in quasar spectra.

Quasar absorption spectra are, however, not only restricted to revealing the presence of discrete clouds of gas along the line of sight. An important open question is whether there exists a truly diffuse intergalactic medium (IGM) that fills the voids between the galaxies and the Lyman forest clouds. If the IGM contained an appreciable density of neutral hydrogen, the redshift-smearred Ly- α absorption in this smoothly distributed intercloud gas would give rise to a 'Gunn-Peterson' continuous absorption trough⁷ on the short-wavelength side of emitted Ly- α . The fact that little, if any, such absorption (beyond the line absorption provided by the discrete Lyman forest lines) is seen^{8,12}, implies either that the density of the ambient IGM is extremely low, or more likely, that the intergalactic gas is highly ionized.

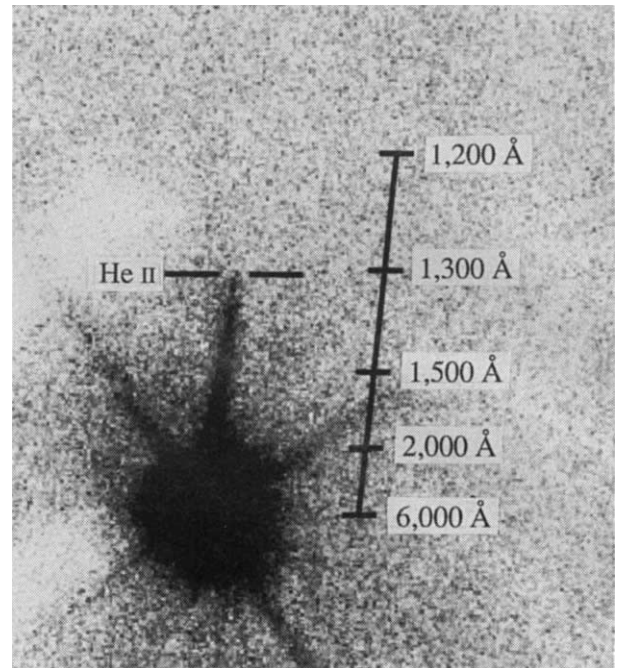
That rather harsh conditions prevailed in intergalactic space at large redshifts is suggested by several properties of the Lyman

forest systems. In particular, the forest clouds are believed to be held highly photoionized (to ~ 1 part in 10^4 in H I) by a metagalactic ionizing background flux, probably arising from the cumulative ionizing output of quasars and young galaxies¹. The strongest evidence for this comes from the 'proximity' effect; this is the tendency of the forest clouds to be under-abundant statistically in the immediate vicinity of quasars^{2,13}. This effect is interpreted as being caused by the extra ionization of the forest clouds introduced by the additional flux from the background quasars themselves. As the latter can be estimated from the brightness of the quasars, the intensity of the metagalactic background—and thereby the baseline ionization level of the clouds—can be gauged from the measured contrast of the effect^{13,14}. That the forest clouds are ionized is also implied by the observed line widths, b , of the forest systems ($b \approx 30 \text{ km s}^{-1}$)^{15,16}: $b = (2kT/m)^{1/2}$, where T is the temperature and m the atomic mass, which indicates a temperature of $T \sim 5 \times 10^4 \text{ K}$ for the forest clouds.

A long-standing^{1,17-23} goal of ultraviolet space astronomy—and the Hubble Space Telescope (HST) in particular—has been to verify directly the high level of ionization of this gas by extending the Gunn-Peterson test to cover the He II 304 Å resonance line of singly ionized helium, and detect the primordial helium that according to Big Bang nucleosynthesis theory²⁴ must be present both in the Lyman forest clouds and in the ambient IGM. If the forest systems are highly ionized in hydrogen, then the helium present at large redshift should also be ionized and therefore mainly detectable in the form of singly ionized He II rather than neutral He I. Because of the very short (extreme ultraviolet) wavelength of the helium resonance lines, such observations can only be carried out above the Earth's atmosphere.

In practice, the task of observing quasars at short extreme ultraviolet rest-wavelengths is complicated by several factors. For example, the He II 304 Å line is only redshifted into the $\lambda \geq 1,200 \text{ Å}$ wavelength range accessible with the HST in the case of the most remote, and therefore faintest, quasars at redshift $z > 3$. Even more important, a typical line of sight out to $z \approx 3$ intercepts (on average) ~ 400 Lyman forest clouds and ~ 6 Lyman limit clouds. The cumulative photoelectric H I absorption, especially in the latter optically thick systems, is extremely effective in completely quenching the flux emitted at He II 304 Å from all but a small minority of all $z > 3$ quasars²⁵.

FIG. 1 The raw co-added far-ultraviolet prism image of Q0302–003 obtained by Faint Object Camera (FOC) on the Hubble Space Telescope (HST). The wavelength scale along the dispersed image and the anticipated position of the He II 304 Å line are indicated. Because the dispersion of the far-ultraviolet prism is a strong function of wavelength, the undispersed visible light gives rise to the intense point-like image at the red end of the spectrum. The diagonal features are diffraction spikes caused by the HST secondary-mirror support spider. The two areas of low background on the left of the image are background 'shadows' cast by the occulting finger in the FOC focal plane in the light of geocoronal Ly- α and the (visible) zodiacal light. The finger appears unfocused because the sub-images were taken at slightly different telescope pointings and were shifted before co-addition.



In the hope of identifying at least one example of a high-redshift quasar, whose intrinsic brightness and atypically clear sight line happen to conspire to make observations of redshifted He II 304 Å absorption feasible, we have been carrying out a survey of known $z > 3$ quasars using the far-ultraviolet objective prism mode of the Faint Object Camera (FOC)²⁶. Before the HST servicing mission, we had obtained exploratory far-ultraviolet prism spectra of a total of 25 objects, which were selected from the literature largely on the basis of their brightness and the appearance of their optical spectra.

With the newly COSTAR-corrected FOC we have now carried out more sensitive follow-up observations of the best (and to date only) 'clear' candidate identified from this survey, the object Q0302–003. Our improved far-ultraviolet spectrum of this $z = 3.286$ quasar reveals what is almost certainly intense He II 304 Å absorption due to foreground intergalactic gas at $z \approx 3.0$ – 3.3 . A remarkable and unexpected finding is that this detected He II absorption is far stronger than anticipated, given the comparative weakness of the matching total absorption seen in the Ly- α line of neutral H I at these redshifts as measured from the ground. In particular, our spectrum of Q0302–003 appears to be completely absorbed on the short-wavelength side of the redshifted He II 304 Å line.

At the modest spectral resolution of our data, we cannot distinguish directly between smooth He II 304 Å absorption from diffuse intergalactic gas and unresolved He II 304 Å line blanketing from the discrete forest clouds. But estimates of the latter contribution, based on the known properties of these systems, indicate that the forest systems alone are unlikely to account for the intense absorption observed. If so, this suggests that we have detected He II 304 Å Gunn–Peterson absorption from the diffuse IGM at $z \approx 3.2$.

Although we can only place a lower limit on the density of intergalactic helium ions required to explain the detected absorption, we argue that the contribution of the absorbing plasma to the cosmological density parameter is not likely to exceed $\Omega \approx 10^{-2}$, and could be as low as $\Omega \approx 10^{-3}$. This uncertainty reflects our lack of knowledge of the ionization level of the detected helium. That the intergalactic helium is found in the form of singly ionized He II, rather than neutral He I, does confirm that intergalactic gas at high redshifts is highly ionized. The inferred high ratio of He II to H I in the absorbing plasma also

provides an important clue as to the possible sources of ionization.

That helium is detected at all in the early Universe also provides a dramatic—but qualitative—confirmation of Big Bang nucleosynthesis theory, according to which a cosmic helium to hydrogen ratio of $\sim 8\%$ (by number) results from processes occurring during the first three minutes of the history of the Universe²⁴.

Q0302–003 revisited

The quasar 0302–003 ($z = 3.286$, visual magnitude, $V \approx 17.8$ mag) was initially chosen for FOC/HST observations on the basis that, aside from an optically thin ($N_{\text{H I}} \approx 2 \times 10^{17} \text{ cm}^{-2}$) Lyman limit system at an absorption redshift $z_{\text{abs}} = 2.53$, its visible spectrum⁵ indicates an unobstructed sight line free of strong absorption systems within the redshift window accessible from the ground. Indeed, the (unpublished) exploratory FOC prism observations of this object taken with the aberrated HST suggested the presence of a weak far-ultraviolet continuum flux extending down to redshifted He II 304 Å, albeit at an intensity of $F_{\lambda} \approx (1-2) \times 10^{-16} \text{ erg s}^{-1} \text{ cm}^{-2} \text{ Å}^{-1}$ at the very limit of the (pre-COSTAR) sensitivity of our survey.

Q0302–003 was accordingly re-observed with the FOC $f/96$ camera and far-ultraviolet prism on 26 January 1994, shortly after the successful installation of the COSTAR instrument and the commissioning of the corrective optics for the FOC. A detailed description of the FOC far-ultraviolet prism and our calibration and reduction techniques has been given in ref. 26 and need not be repeated here. Aside from the corrective optics—and the dramatic factor $\sim 4-5$ increase in sensitivity stemming from the now properly focused HST image—an instrumental configuration identical to that employed previously was used. A total of 10 separate exposures providing an integrated exposure time of 10,659 s were taken, with the HST pointing offset by 0.2 arcsec between exposures in order to move the dispersed images on the FOC detector and minimize residual flat-field effects in the faint ultraviolet spectrum of this object.

The combined raw far-ultraviolet prism image of Q0302–003 obtained by aligning and co-adding the individual FOC sub-exposures is shown in Fig. 1. The corresponding extracted flux and wavelength calibrated one-dimensional spectrum derived

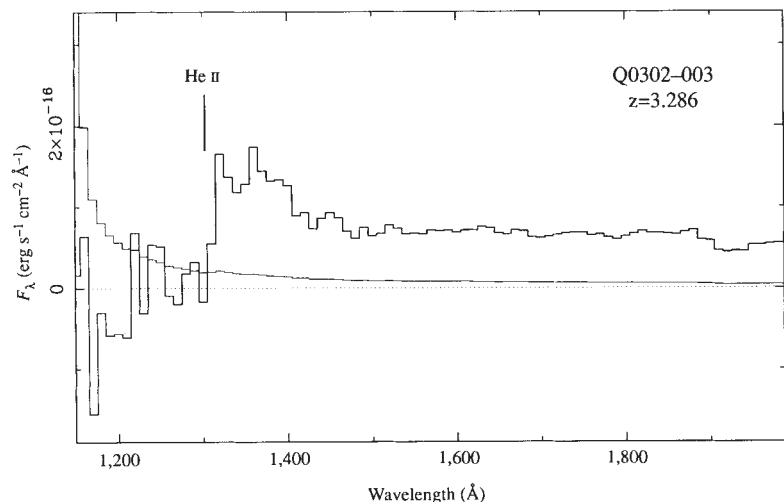


FIG. 2 Flux (F_λ) and wavelength calibrated FOC far-ultraviolet prism spectrum (solid line) of Q0302-003. The thin solid line gives the 1σ uncertainty per $\Delta\lambda=10$ Å wavelength bin due to photon statistics. The absolute flux calibration should be accurate to within a factor of ~ 2 . The position of the He II 304 Å line in the quasar rest frame is marked.

from our data is shown in Fig. 2. Marked in both figures is the anticipated $\lambda=1,303$ Å position of the He II 304 Å line, given the $z=3.286$ redshift of Q0302-003. It is evident that the spectrum comes to an abrupt end just on the short-wavelength side of this wavelength, below which no obvious signal is detected. The mean flux measured from our data within a $\Delta\lambda=50$ Å wavelength bin just below the edge is

$$F_\lambda \approx 0.05 \pm 0.11 \times 10^{-16} \text{ erg s}^{-1} \text{ cm}^{-2} \text{ Å}^{-1}$$

which is statistically consistent with zero flux at the 1σ level. The equivalent continuum flux measured just on the long-wavelength side of the edge is

$$F_\lambda \approx 1.25 \pm 0.09 \times 10^{-16} \text{ erg s}^{-1} \text{ cm}^{-2} \text{ Å}^{-1}$$

The corresponding flux ratio ($f \approx 0.04 \pm 0.09$) implies that the effective optical depth across the edge is very high: $\tau_{\text{He II}} = -\ln f \approx 3.2^{+0.4}_{-0.1}$. The 90% confidence bound on this quantity inferred from our data is $1.7 < \tau_{\text{He II}} < \infty$.

In addition to the edge, the only other features apparent in our spectrum are the two possible emission lines at $\lambda \approx 1,320$ Å and $\lambda \approx 1,360$ Å. Whereas the former is probably the unabsorbed red wing of the He II/O III 304 Å emission line, we have found no obvious identification for the latter feature ($\lambda \approx 320$ Å rest wavelength).

Before the detected absorption edge can be unequivocally identified with intergalactic He II absorption, we need to consider whether this feature could possibly be due to a freak coincidence involving an H I Lyman limit system, whose redshift happens to make its Lyman edge coincident with the redshifted wavelength of the He II 304 Å line. Given the $\Delta\lambda \approx 10$ –20 Å accuracy of our wavelength scale, such a system would have to fall within a narrow redshift window of width $\Delta z \approx 0.03$ centred on the redshift $z_{\text{abs}} \approx 0.43$. Also, the column density of the system would have to be $N_{\text{H I}} \geq 1 \times 10^{18} \text{ cm}^{-2}$ in order to absorb the flux down to $\lambda \approx 1,200$ Å. Although our spectrum is of too low resolution and signal-to-noise ratio to constrain significantly the presence of the Ly- α and Ly- β lines expected of such a system (anticipated equivalent widths $W_\lambda \approx 3$ Å), the available optical spectra^{5,13,27} of Q0302-003 do not reveal any obvious metal lines near this redshift. In particular, no obvious strong Mg II doublet is present near $\lambda \approx 4,000$ Å (although this wavelength falls in the dense Lyman forest); nor is any Ca II doublet apparent near $\lambda \approx 5,640$ Å in the red.

Based on the observed statistics of Lyman limit systems^{3,6}, it can be shown that the *a priori* probability of encountering a Lyman limit system of sufficient column density within the critical redshift window is of the order of $P \approx 2\%$. Although this

probability is not negligible, we interpret the fact that the drop detected in our spectrum coincides with the expected position of the redshifted He II 304 Å line (to within the 10–20 Å accuracy of our wavelength calibration) to mean that we are indeed seeing absorption due to intergalactic He II.

Characteristics of the ionized helium

The intervening He II ions responsible for the detected He II 304 Å absorption are almost certainly situated either in the Lyman forest clouds or in the ambient IGM; that is, in the two most likely examples of primordial matter in the early Universe¹. The fact that we have detected helium in this gas at very early epochs corresponding to redshifts $z \approx 3.0$ –3.3 provides an important, albeit qualitative, verification of Big Bang nucleosynthesis theory²⁴. All previous attempts at detecting He Gunn–Peterson absorption in the He I 584 Å line of neutral helium have been unsuccessful^{28–30}; helium absorption in quasars has only previously been detected³¹ in the form of weak He I lines in the case of three metal-line systems in the ultraviolet-bright object HS1700+6414 ($z=2.72$).

That the intervening helium reveals itself strongly in the form of singly-ionized He II also lends support to the notion that the hydrogen contained in the Lyman forest clouds—and by implication the ambient IGM—is highly ionized, as implied by the proximity effect and the forest line widths.

The quantitative interpretation of our data is not entirely straightforward. Our lack of knowledge of the precise ionization level of the detected He II makes an accurate determination of the helium abundance of the absorbing gas impossible at present. Moreover, at our low spectral resolution of $\Delta\lambda \approx 20$ Å, we are not able to distinguish between the He II absorption due to line blanketing in the discrete He II lines corresponding to the Lyman forest clouds and the redshift-smeared He II Gunn–Peterson trough of a more smoothly distributed IGM.

Diffuse gas or discrete clouds?

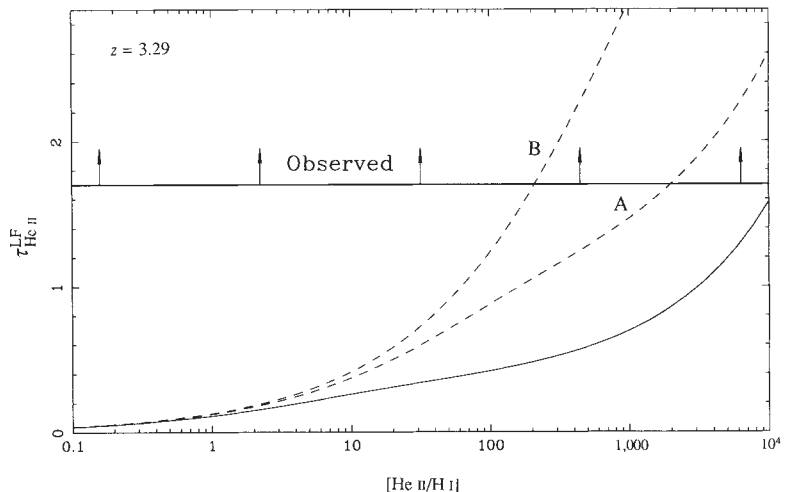
The optical depth of a Gunn–Peterson He II 304 Å absorption trough in an $\Omega=1$ universe is given by⁷

$$\tau_{\text{He II}}^{\text{GP}}(z) = \left[\frac{c}{H_0} \right] n_{\text{He II}}(z) \sigma_I (1+z)^{-3/2} \quad (1)$$

where $H_0 = 100 h \text{ km s}^{-1} \text{ Mpc}^{-1}$ is the Hubble constant, $\sigma_I = \lambda_I (\pi e^2 / m_e c^2) f_{ij} = 1.1 \times 10^{-18} \text{ cm}^2$ the integrated cross-section of the He II 304 Å transition, and $n_{\text{He II}}(z)$ is the volume density of He II ions at the appropriate redshift.

The corresponding wavelength-averaged contribution to

FIG. 3 Predicted wavelength-averaged optical depth due to He II 304 Å line blanketing in Lyman forest systems ($\tau_{\text{HeII}}^{\text{LF}}$) as a function of the assumed He II to H I ratio by number ($[\text{He II}/\text{H I}]$). The solid curve gives the net blanketing due to known forest systems having H I column densities $N_{\text{HI}} > 10^{13} \text{ cm}^{-2}$. The two dashed curves give the blanketing assuming that the observed distribution in H I column density can be extrapolated down to $N_{\text{HI}} \approx 10^{12} \text{ cm}^{-2}$ (curve A) and $N_{\text{HI}} \approx 10^{11} \text{ cm}^{-2}$ (curve B). The 90% confidence limit of $\tau_{\text{HeII}} > 1.7$ for the total He II 304 Å absorption derived from our data is also shown as the line marked 'observed'.



$\tau_{\text{HeII}}^{\text{LF}}$ from He II line blanketing in the Lyman forest systems can be written^{25,32}

$$\tau_{\text{HeII}}^{\text{LF}}(z) = \frac{dN(z)}{dz} \frac{\langle W_\lambda \rangle}{\lambda_l} (1+z) \quad (2)$$

where dN/dz is the number of forest lines per unit redshift and $\langle W_\lambda \rangle$ is the mean He II 304 Å equivalent width of the forest systems averaged over their distribution in column density.

As stated above, the conservative lower limit on the total optical depth of the redshift-smeared He II 304 Å absorption inferred from our data is $\tau_{\text{HeII}} > 1.7$. In comparison, the analogous H I Ly- α -induced depression measured in quasar spectra just on the short-wavelength side of emitted Ly- α at $z \approx 3.3$ is far smaller^{8–10} and equivalent to $\tau_{\text{HI}} \approx 0.35 \pm 0.10$. This amount of absorption can, to within the uncertainties, be accounted for^{8–12} by Ly- α line blanketing in the known population of Lyman forest systems with column densities in the measurable range $10^{13} \text{ cm}^{-2} \leq N_{\text{HI}} \leq 10^{17} \text{ cm}^{-2}$ —thereby leaving little or no room for a contribution from a smooth IGM ($\tau_{\text{HI}}^{\text{GP}} \lesssim 0.05$). A similar situation almost certainly does not hold in the case of the far stronger He II absorption reported here. The solid line in Fig. 3 shows the expected He II 304 Å line blanketing due to the known population of Ly- α forest systems, calculated from equation (2) assuming that all forest clouds have a constant He II to H I ratio $[\text{He II}/\text{H I}]$. A forest cloud column-density spectrum, normalization and redshift evolution identical to that in ref. 25 was used, and a thermal line width of $b = 15 \text{ km s}^{-1}$ (corresponding to a temperature of $T \approx 5 \times 10^4 \text{ K}$) was assumed when calculating the equivalent widths of the He II lines.

Although the matching forest blanketing in H I Ly- α calculated for our choice of parameters is $\tau_{\text{HI}}^{\text{LF}} \approx 0.29$, and in good agreement with the observed values, it is clear from Fig. 3 that the observed He II 304 Å absorption is significantly stronger than can be accounted for by He II line blanketing in the measured population of Lyman forest clouds over nearly the entire range of plausible values^{1,22,26} for $[\text{He II}/\text{H I}]$. The reason for this is merely that the blanketing is determined by the equivalent widths of the lines, which are subject to saturation at moderate-to-large column densities. Large values of $\tau_{\text{HeII}}^{\text{LF}}$ are only possible for very high values of $[\text{He II}/\text{H I}]$ —corresponding to the extreme case where even the weakest assumed forest systems with H I column densities $N_{\text{HI}} \approx 10^{13} \text{ cm}^{-2}$ are damped in He II 304 Å and all He II lines are on the square root part of the curve of growth.

Given the conclusion that the anticipated line blanketing in He II 304 Å from the known population of Lyman forest systems is $\tau_{\text{HeII}}^{\text{LF}} \lesssim 0.5$ for all but the most extreme values of $[\text{He II}/\text{H I}] \geq 10^3$, it is very tempting to attribute the bulk of the detected attenuation to He II Gunn–Peterson absorption from the ambi-

ent IGM. However, other possibilities need to be explored before we can claim with confidence to have detected the long-sought-after IGM.

One way that the Lyman forest blanketing could approach our observed limit on τ_{HeII} for more moderate values of $[\text{He II}/\text{H I}]$, is if there exists an abundant population of forest systems with column densities $N_{\text{HI}} \lesssim 10^{13} \text{ cm}^{-2}$ well below the present Ly- α detection threshold. As specific examples, we show as dashed lines in Fig. 3 the He II forest blanketing calculated as above, but assuming that the $\propto N_{\text{HI}}^{-1.5}$ column density distribution of the forest systems can be extrapolated beyond $N_{\text{HI}} \approx 10^{13} \text{ cm}^{-2}$ down to $N_{\text{HI}}^{\text{min}} \approx 10^{12} \text{ cm}^{-2}$ and $N_{\text{HI}}^{\text{min}} \approx 10^{11} \text{ cm}^{-2}$. Because the added systems have such small equivalent widths in H I, the Ly- α blanketing predicted for these models is increased only slightly to $\tau_{\text{HI}}^{\text{LF}} \approx 0.35$ and $\tau_{\text{HI}}^{\text{LF}} \approx 0.36$, and is still consistent with the observational constraints. The addition of these low-column systems does, however, lead to a dramatic increase in the He II absorption for $[\text{He II}/\text{H I}] \geq 10^2$.

Whether such a numerous population of weak Lyman forest lines exists or not is not obvious, as there is some evidence^{10,15} that the forest cloud spectrum starts to turn over already at $N_{\text{HI}} \lesssim 5 \times 10^{13} \text{ cm}^{-2}$. But given the large filling factor that such a population of small clouds would have to have (~ 5 –50% using standard parameters), it is to some extent a question of definition whether such an abundant population of rarefied clouds should be classified as belonging to the ambient IGM or not.

Density and ionization of the intergalactic gas

For the sake of simplicity, we assume in the following that the diffuse plasma giving rise to the required extra He II absorption is indeed the elusive IGM—keeping in mind that this gas probably possesses a wispy cloud-like structure. As equations (1) and (2) are equivalent in the limit where all the forest lines are optically thin, we can gauge the minimum total amount of intergalactic He II in the forest clouds and IGM required to explain our observations by applying equation (1) to the total absorption. Our conservative limit of $\tau_{\text{HeII}} > 1.7$ thus translates to a lower limit on the volume-averaged He II particle density of $\langle n_{\text{HeII}} \rangle > 1.5h \times 10^{-9} \text{ cm}^{-3}$ at $z = 3.29$. In order to convert this He II density into a limit on the total cosmological density parameter of the absorbing gas, we need to know both the relative abundance and ionization level of the helium:

$$\Omega_{\text{IGM}} > 1.7h^{-1} \times 10^{-6} \left[\frac{\text{He II}}{\text{He}} \right]^{-1} \left(4 + \left[\frac{\text{He}}{\text{H}} \right]^{-1} \right) \quad (3)$$

For a standard Big Bang helium to hydrogen abundance (by number) of $[\text{He}/\text{H}] \approx 0.08$, equation (3) reduces to $\Omega_{\text{IGM}} > 2.8h^{-1} \times 10^{-5} [\text{He II}/\text{He}]^{-1}$.

Most theories for the ionization of the IGM^{1,18,20} assume high levels of ionization of both the hydrogen and the helium that is contained in the Lyman forest clouds and the surrounding medium: $[\text{He III}/\text{He}] \approx 1$, $[\text{He II}/\text{He}] \lesssim 10^{-2}$. In this case our observations would imply a density of $\Omega_{\text{IGM}} \gtrsim 10^{-3}$. But the notion of He II being a minority ionization species is very difficult to reconcile with both the strength of the He II opacity reported here and the extremely low limits on the intergalactic H I density placed by the classical Ly- α Gunn-Peterson test. Specifically, in the favoured scheme of an IGM and Lyman forest photoionized by a metagalactic background flux, the $[\text{He II}/\text{H I}]$ of the gas is determined solely by the shape of the input spectrum^{1,20,22,26}. In the canonical case³³ of an energetic quasar-like power-law spectrum in frequency, $F_\nu \propto \nu^{-\alpha}$, having spectral index $\alpha \approx 1$ and extending well below the photoionization edge of He II, one expects $[\text{He II}/\text{H I}] \approx 10$. In contrast, our observed limit of $\tau_{\text{He II}} > 1.7$ in He II at $z \approx 3.29$, when combined with the equivalent limit for H I of $\tau_{\text{H I}}^{\text{GP}} \lesssim 0.05$, implies $[\text{He II}/\text{H I}] \approx 4\tau_{\text{He II}}^{\text{GP}}/\tau_{\text{H I}}^{\text{GP}} \gtrsim 10^2$. This corresponds to a far softer spectral slope of $\alpha \gtrsim 3$ —that is, a spectrum much less capable of ionizing He II. In other words, in view of the intensity of the detected He II absorption, and the weakness of the corresponding absorption in H I (and He I), it seems highly likely that the bulk of the intergalactic helium present at $z \approx 3.3$ is in the form of singly ionized He II, rather than doubly ionized He II (or neutral He I). In this case where $[\text{He II}/\text{He}] \approx 1$, the absorption detected in our spectrum requires a more modest minimum IGM density corresponding to $\Omega_{\text{IGM}} \gtrsim 10^{-5}$.

The source of ionization?

In the photoionization model, a steep ionizing continuum is expected if young galaxies are significant contributors to the ionizing background³⁴. The combination of a high value of $[\text{He II}/\text{H I}]$ and $[\text{He II}/\text{He}] \approx 1$ could also arise in the quasar-dominated scheme if the integrated ionizing flux for some reason does not extend below 54 eV, and is therefore capable of ionizing hydrogen and helium only once. That the actual ionizing background at $z \approx 3.3$ —regardless of its source—must drop significantly in intensity across the He II photoionization edge is evident from the intensity of the detected absorption in

He II 304 Å, which implies an even greater opacity at $\lambda \leq 228$ Å rest wavelengths.

The inference that the Universe is opaque in He II at $z \approx 3.3$ raises the intriguing possibility that the observed high value of $[\text{He II}/\text{H I}]$ is merely a consequence of the IGM not yet having become re-ionized in He II at this epoch. In the conventional^{12,35} scheme, where the ionizing flux from quasars is responsible for re-ionizing the IGM through photoionization, complete re-ionization and overlapping of the individual H II (and He II) Strömgren spheres surrounding quasars is expected to occur at $z \gtrsim 5$. But because of the lower output of He II ionizing photons compared to H I and He I ionizing photons, the re-ionization of the Universe in He II could conceivably lag behind that of H I and He I by a significant interval in redshift^{21,36}. One way of checking this hypothesis is to carry out similar observations of quasars at lower redshifts ($2 < z < 3$) with space-borne instrumentation covering shorter ultraviolet wavelengths. Because the quasar population peaks at $z \sim 2$, the He II re-ionization is expected to also have occurred by this epoch, resulting in much weaker He II 304 Å absorption. Mapping the redshift evolution of the He II absorption in this manner would obviously yield important clues as to the nature and the history of the intergalactic gas.

In this context it should be noted that although our objective prism spectrum may be relatively crude, and although we are not able to distinguish between He II line blanketing and true Gunn-Peterson absorption, the goal of obtaining significantly higher resolution and signal-to-noise ratio observations of the He II forest region in any quasar poses a considerable technical challenge to both existing and future space instrumentation. Although a few further examples of ‘clear’ $z > 3$ quasars are likely to be found in the future³⁷, these are not likely to be significantly brighter than Q0302–003. Similarly, even though several brighter candidates for He II observations at $2 < z < 3$ are already known^{25,26}, the sensitivity of presently foreseen instrumentation covering the technically difficult $\lambda < 1,200$ Å range is also correspondingly lower in comparison with that of HST at longer ultraviolet wavelengths. Considerable effort and technical skill will be required to address the many questions raised by this first glimpse of intergalactic He II 304 Å absorption in quasars. □

Received 6 April; accepted 7 June 1994.

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ACKNOWLEDGEMENTS. We thank the many dedicated individuals of NASA, ESA, STScI, and US and European industry who made the first HST servicing mission—and the COSTAR instrument in particular—a success. Special thanks are due to P. Stanley and other members of the operations and planning teams at STScI for assuring that our observations were executed early while seasonably still possible. P.J. thanks colleagues at Johns Hopkins University for hospitality during the COSTAR/FOC commissioning campaign; P.G. and R.J. acknowledge support from ESA. This work is based on observations with the NASA/ESA Hubble Space Telescope, obtained at the Space Telescope Science Institute, which is operated by AURA, Inc., under NASA contract.

The age of the Veritas asteroid family deduced by chaotic chronology

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ASTEROID families are groups of objects produced in disruptive collisions of a parent body. Although family members are widely dispersed in real space, they cluster in the parameter space defined by their so-called proper elements, and can thus be distinguished from the background asteroid population¹⁻³. For most asteroids, these parameters are very close to being invariants of motion and families are still apparent billions of years after their formation^{4,5}. But these parameters undergo chaotic diffusion, and in some cases the rate of diffusion might be large enough that a family member exits from the region of proper-element space occupied by the family after a characteristic time which is shorter than the lifetime of the Solar System. In this case, the characteristic time should provide an approximate upper bound to the age of the family. Here we use this 'chaotic chronology' method to estimate the lifetime of the unusually compact Veritas family. Calculations of the evolution of the proper elements of the family show that two members (including the largest, 490 Veritas) wander outside the borders of the family on a timescale of about 50 Myr, indicating that the family has an age of less than this.

After a time $>10^5$ yr since the formation of an asteroid family from the breakup of its parent body, the osculating (that is, instantaneous) orbital elements of the fragments become widely dispersed. Family members can, however, still be discriminated from background asteroids by deriving some quantities characterizing the orbits and varying very slowly with time. These quantities, called proper elements, are defined as integrals of suitable truncations of the full N -body dynamical equations which describe the motion of the asteroids under the gravitational perturbations of the major planets. A proper semimajor axis a , a proper eccentricity e and a proper inclination I can thus be calculated as a function of the osculating elements by means of a generalized averaging technique^{2,5,7}, in such a way that they change by $\leq 1\%$ of their value over $\sim 10^7$ yr. In this way some 20 asteroid families, with $>2,000$ member bodies, have been reliably identified by cluster analysis in the proper element space^{3,4,8}. But because proper elements are almost constant in time, this method provides no clues about family ages. On the other hand, asteroid collisions are a continuing process, so some asteroid families have been probably formed comparatively recently. We have found a way to identify some of these 'young' families, and to derive a rough upper limit on their age.

The key argument to be used is that proper elements are not exactly constant, because the full gravitational N -body problem (with one asteroid attracted by the Sun and by the major planets) has no exact integral⁹. The phase space of such a problem must necessarily contain an everywhere dense set of chaotic orbits, characterized by a positive Lyapunov exponent χ (namely by the divergence of two infinitesimally nearby orbits as a function of time t at a rate proportional to $\exp(\chi t)$), and also by the nonexistence of a Kolmogorov-Arnold-Moser invariant torus on which some proper elements are exactly constant^{10,11}. The best known cases of chaotic dynamics in the Solar System correspond to very unstable orbits, such as those occurring in the Kirkwood gaps¹² and for the planet-crossing asteroids¹³. In these cases, large and random changes in the orbits occur in a time

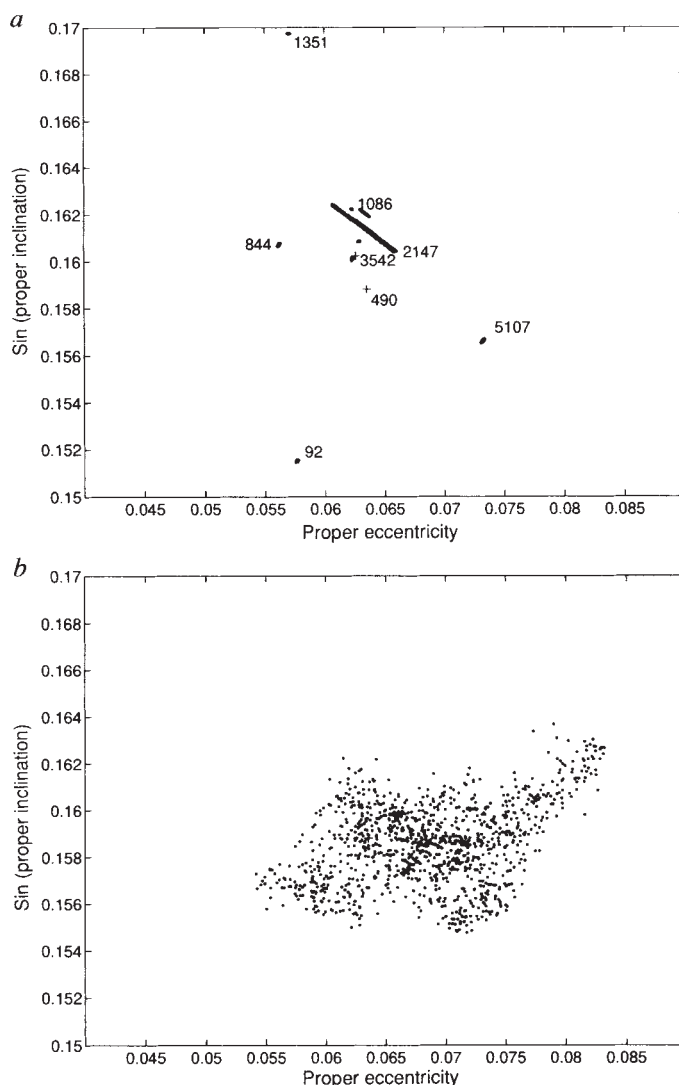


FIG. 1 *a*, Proper eccentricity (e) and (sine of) proper inclination (I) over the past 72 Myr for five Veritas family members (asteroids 1086, 2147, 2428, 2934 and 3090; the latter three correspond to the three small unlabelled spots near the centre of the family) and four non-family asteroids (92, 844, 1351 and 5107) with relatively stable proper elements. Along the third dimension not displayed in this plot (that is, the proper semimajor axis), all these orbits are very stable, with r.m.s. changes $\leq 8 \times 10^{-5}$ AU. For the two strongly chaotic members 490 and 3542, only their present positions are plotted (with a cross). The only 'stable' asteroid displaying significant variations of the proper elements is 2147, but it stays within the boundaries of the family. *b*, Each dot in this plot represents the proper elements of 490 Veritas once every 60,000 yr, over the past 72 Myr. The 'Veritas cloud' extends clearly beyond the region occupied by the family, with r.m.s. changes $\approx 6 \times 10^{-3}$ for e and $\sim 22 \times 10^{-3}$ for $\sin I$. Asteroid 3542 behaves in a similar way. Both these orbits have a semimajor axis slightly larger than the other family members, and thus are strongly affected by the 21:10 resonance with Jupiter; the semimajor axes undergo changes with r.m.s. $\sim 7 \times 10^{-3}$ AU, but with no long-term trend.

of the order of the Lyapunov time $T_L = 1/\chi$, so that fragments from an asteroid impact would be scattered beyond recognition in a large region (that is, the entire Solar System, and even beyond) in a time much shorter than the expected age of the youngest families. Thus the search for asteroid families still preserving, in the distribution of the orbits, evidence for the formation event, has to be limited to the examples of stable chaos¹⁴. This type of chaos affects many orbits in the densely populated regions of both the main belt⁵ and the Trojan clouds (ref. 15;

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A.M., A. M. Nobili and Z. Knežević, manuscript in preparation) and is characterized by relatively short Lyapunov times (say, $<10^5$ yr), but much longer timescales for significant changes of proper elements. In the presence of stable chaos, provided that the time span over which the proper elements change—by an amount corresponding to the size of the zone occupied by the family—is within the range of likely family ages ($\sim 10^6$ to $\sim 10^9$ yr)¹⁶, it is possible to estimate the age itself. Thus for a dense family, with a proper element dispersion corresponding to the extent of chaotic wandering over a time span Δt , the age must be $\leq \Delta t$. This estimate is only reliable in a statistical sense, because a chaotic diffusion-like evolution is an essentially random process.

We have investigated the most densely clustered among the asteroid families reliably identified in recent studies^{4,8}, by performing accurate numerical integrations of the orbits of a number of asteroids (under the perturbations of the major outer planets), going backwards from now to 72 Myr ago. The output has been processed to generate very accurate proper elements with a combination of numerical and analytical methods. First, the output has been filtered digitally¹⁷ to remove planetary perturbations with periods $\leq 6,000$ yr; second, proper elements have been computed analytically with the algorithm by Milani and Knežević⁵; finally the proper elements have again been filtered to remove the remaining secular changes with periods up to 60,000 yr (in some cases up to 300,000 yr). In most cases we have found the diffusion to be much smaller than the size of the family; for example, this is the case for the Hoffmeister, Dora and Adeona families. So far we have found only one family for which this is not the case.

The asteroid 490 Veritas is the largest member of a family in the outer portion of the main belt, close to the inner side of the 2:1 mean motion resonance with Jupiter. The family is so compact with respect to the density of nearby background asteroids that one can estimate⁴ that the *a priori* probability of having one and two chance interlopers in it does not exceed 3% and 0.1%, respectively. We have calculated the orbits of the seven numbered asteroids rated as members of the family^{4,8}, plus four nearby background asteroids, from the present to $t = -72$ Myr. The orbits of the two family members 490 and 3542 have been found to be highly chaotic, with a Lyapunov time $T_L \approx 10,000$ yr; the other family members are weakly chaotic, with $100,000$ yr $\leq T_L \leq 3$ Myr; the nearby background asteroids we have tested have $T_L \geq 1$ Myr. Most family members experience remarkably stable chaos: the proper elements e and $\sin I$, calculated by our mixed procedure, undergo changes with r.m.s. $\leq 10^{-4}$ (Fig. 1a); only asteroid 2147 displays a long periodic oscillation of the order of 10^{-3} . On the other hand, the proper e and $\sin I$ of asteroids 490 and 3542 undergo much larger changes (Fig. 1b); in 72 Myr they wander in a region much larger than the one spanned by the family, as can be appreciated by comparing Fig 1a and b. The instability of the proper elements results from temporary captures into high-order mean motion resonances with Jupiter, the main one being the 21:10. An intermittent behaviour arises because of the coupling between mean motion resonances and secular perturbations; theories accounting for such complicated effects are still under development (ref. 18; A.M., A. M. Nobili and Z. Knežević, manuscript in preparation). Although the orbits switch between different resonant states, the semimajor axes do not undergo secular variations, and both the exponential divergence and the instability of proper e and $\sin I$ appear to be permanent features (at least over 72 Myr).

To understand the implications for the history of the Veritas asteroid family, we have plotted the distance, in the d_1 metric of Zappalà *et al.*^{1,4} (see Fig. 2 legend for details), from a family member whose proper elements stay almost constant (1086 Nata) to the other 10 orbits, over the past 72 Myr (Fig. 2). Some family members (2428, 2934 and 3090) remain always very close to 1086; asteroid 2147 is sometimes less close, but comes back

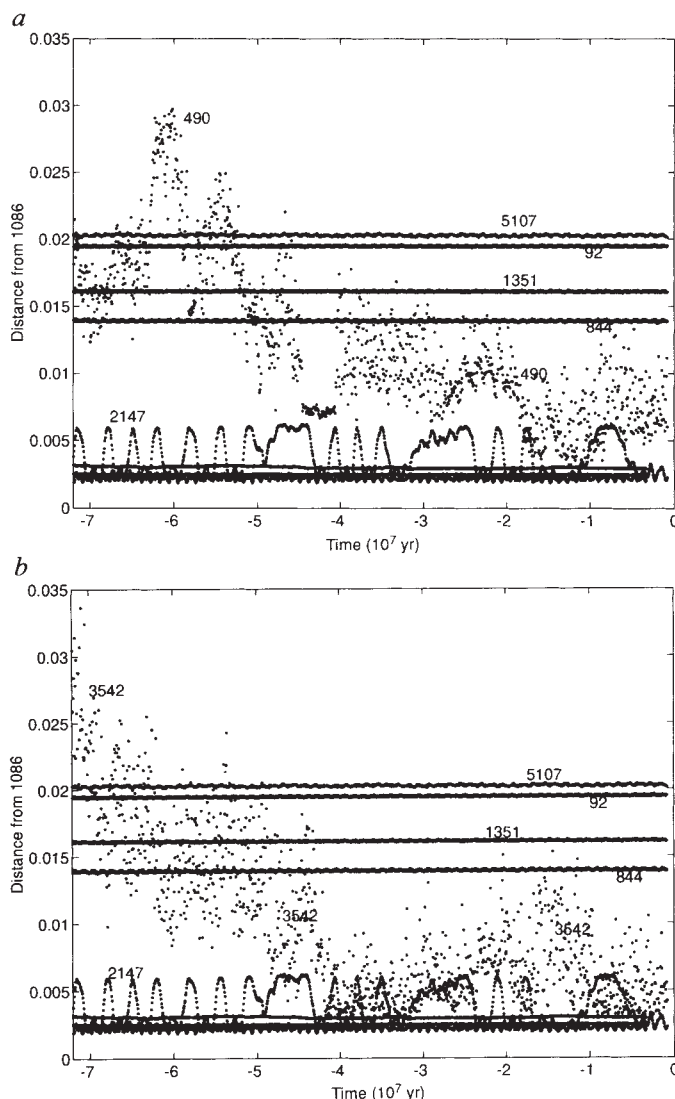


FIG. 2 Time dependence of the distance in the proper-element space between asteroid 1086 (a relatively stable Veritas family member) and the other asteroids we have integrated (note that $t=0$ corresponds to the present day). The distance is defined from the proper element differences through the metric $d_1 = (1.25(\delta a/a)^2 + 2(\delta e)^2 + 2(\delta \sin i)^2)^{1/2}$, according to Zappalà *et al.*^{4,8}. The three asteroids 2428, 2934 and 3090 (corresponding to the lowest, unlabelled lines) have the smallest, almost constant distances of ~ 0.003 (corresponding to ~ 50 m s⁻¹ in relative velocity), and are family members. So is 2147, although it reaches distances up to ~ 0.006 because of the long periodic oscillation already seen in Fig. 1a. On the contrary, asteroids 92, 844, 1351 and 5107 are always too far away ($0.014 \leq d_1 \leq 0.021$) to be plausible family members. The chaotic behaviour of 490 (a) and 3542 (b) is such that during $0 \geq t \geq -50$ Myr they are most of the time within $d_1 \leq 0.01$ from asteroid 1086 (a significant fraction of the time even within $d_1 \leq 0.008$), while for $-50 \text{ Myr} \geq t \geq -72$ Myr they wander in a region with a distance from 1086 up to $d_1 = 0.03$ for 490 (even more for 3542). Thus these two objects stay close enough to the other members to be classified in the family for most of the last 50 Myr, but not before.

often. The background objects are always at a much larger distance. Asteroids 490 (Fig. 2a) and 3542 (Fig. 2b), which are currently close enough to be in the family, stay at distances consistent with family membership for most of the past 50 Myr, but not before. Thus, if the clustering procedure used to define the family membership were applied with the proper elements calculated before -50 Myr, asteroids 490 and 3542 would be rated as background objects.

Chaotic motions being, by definition, unpredictable, it is not possible to prove that asteroids 490 and 3542 have not been

together near the other family members at some time in the remote past. But the formation of the family during one of these previous returns appears quite unlikely. Over the span of our integrations, the area occupied by the current family members in the proper e , $\sin I$ plane increases by a factor of ~ 10 , because of the chaotic behaviour of 490 and 3542. Both asteroids are likely, over a much longer interval, to diffuse in a larger region. By the so-called ergodic principle, if 490 and 3542 were bound to wander at random inside this larger region for all the time in the past, they would spend together in the current family region only $<1\%$ of the Solar System's lifetime. Therefore we conclude that the Veritas family has been probably formed by a collision which occurred in the past 50 Myr. This number is only a rough estimate; because of the unpredictable nature of chaotic orbits, another experiment with minute changes in the initial conditions could result in the transition out of some resonant state, as happened to asteroid 490 around 44 Myr ago, taking place at some other time. However, the order of magnitude of the estimate is reliable, as it is confirmed by the remarkably similar behaviour of 490 and 3542.

The evidence from the available physical observations of these asteroids appears consistent with the dynamical argument. Five family members have known albedos and diameters, derived from the Infrared Astronomical Satellite (IRAS) survey¹⁹. The diameters are 116 and 66 km for 490 and 1086, respectively, and between 25 and 30 km for 2147, 2428 and 2934. This suggests that the family is the outcome of the catastrophic disruption of a parent body of diameter ≈ 150 km. 490 Veritas is a C-type asteroid²⁰ with an albedo of 0.06; for the other four objects the type is unknown, but the albedos are all between 0.04 and 0.09. Note that $\sim 74\%$ of the IRAS-measured non-family asteroids with $3 < a < 3.3$ AU (astronomical unit, AU), have albedos < 0.09 , so the *a priori* probability that five unrelated IRAS-observed objects were all so dark would be just 22%. This provides some support for the reliability of the family, but a positive confirmation of the genetic relationship among the family members from physical data would require further observations. The nearby asteroid 92 Undina is an M-type object (126 km across) with an albedo of 0.25, and therefore is likely to be a background asteroid unrelated to the Veritas family (92 has been classified by some authors^{21,22} as a member of the family, but lying at the very periphery of it). This indicates that objects at a distance $d_i \approx 0.02$ from 1086 should not be considered as family members, whereas 490 and 3542 have been identified as such because they are now (and have been for most of the past 50 Myr) significantly closer to each other and to the other members.

Finally, let us discuss the likelihood of the existence of a family as young as 50 Myr and with such a large parent body. To shatter a 150-km asteroid, with the typical inter-asteroidal impact velocity of ~ 5 km s⁻¹, a projectile > 30 km in diameter is needed^{23,24}. In the asteroid belt there are some 1,500 such projectiles; by using the known collision probabilities²⁵, the lifetime before catastrophic disruption of a 150-km body can be estimated to be 3×10^{10} yr. With ~ 100 targets of this size available in the current belt, such an event should occur once every 300 Myr, consistent with the ~ 10 such families which have been detected⁴. Thus our estimate for the age of the Veritas family indicates that this was a comparatively recent disruption event, possibly the most recent one involving a target asteroid of size ≥ 150 km. $\square$

Received 11 January; accepted 2 June 1994.

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Transfer of methylene groups promoted by metal complexation

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SELECTIVE chemical transformations involving hydrocarbons are a major goal in synthetic chemistry. Transition-metal complexes are capable of promoting a range of selective transformations of organic molecules under mild conditions^{1,2}, and much effort has been devoted to studying their reactivity towards hydrocarbons^{3–7}. Here we report a reaction promoted by a rhodium complex in which a methylene (CH₂) group can be abstracted from a methyl (CH₃) group and inserted into a variety of bonds, such as Si–H, Si–Si and C–H. Our approach presently requires a certain coordination geometry in the metal complex, involving chelation of the metal centre, but we believe that it can be extended to other substrates capable of metal coordination and may eventually lead to a general strategy for CH₂ transfer from hydrocarbons to other molecules under mild conditions.

The ability to insert a metal into an unactivated carbon–carbon bond⁷ raises an intriguing possibility in which a hydrocarbon serves as a source of CH₂ groups. We hypothesized that if the inserting transition metal centre is capable of binding and activating an additional substrate, the result could be selective insertion of the CH₂ group into another chemical bond (Fig. 1).

On reaction of an alkyl group with a metal hydride, the kinetically favourable C–H activation process can lead to an alkyl–metal complex with H₂ liberation and no change in metal oxidation state. Oxidative addition of an A–B molecule, followed by reductive elimination would generate a C–A bond. If metal insertion into C–C were to take place, an M–CH₂A moiety would result. Coupling of this moiety with the metal-bound B fragment would result in overall CH₂ transfer to form A–CH₂–B.

We have validated this hypothesis in a number of cases (Fig. 2). Reaction of compound **1a** with HRh(P(C₆H₅)₃)₄ leads to complex **2a**, as we have previously reported⁷. Similarly, reaction of **1b** with HRh(P(C₆H₅)₃)₄ leads to complex **2b**, which was fully characterized. Remarkably, heating of **2a** with HSi(OC₂H₅)₃ at

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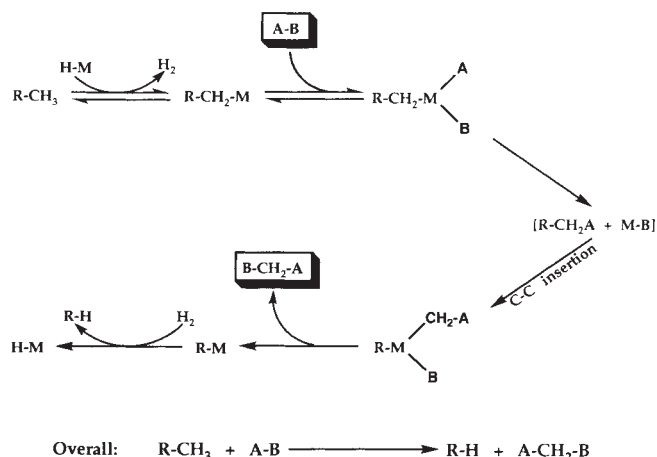


FIG. 1 Hypothesis for methylene group transfer from hydrocarbons (see text; R, alkyl or aryl group).

100 °C leads to quantitative formation of $\text{CH}_3\text{Si}(\text{OC}_2\text{H}_5)_3$, which was unambiguously characterized by ^1H , ^{13}C and ^{29}Si NMR and gas chromatography-mass spectrometry (GC-MS). Complex **3a** is also quantitatively formed, from which the 'demethylenated' arene **4a** can be released (Fig. 2). Thus, transfer of a CH_2 group from carbon to silicon was accomplished, showing that functionalization of an unstrained carbon-carbon single bond by hydrosilylation is possible. Hydrosilylation of carbon-carbon multiple bonds is a reaction of importance both in industry and the laboratory, used for the production of organosilicon compounds, such as adhesives, binders, coupling agents and biologically active compounds⁸. Although this family of reactions has been studied for almost half a century, to the best of our knowledge hydrosilylation of unstrained carbon-carbon single bonds has not been previously reported.

A similar reaction, although slower, takes place with $\text{HSi}(\text{C}_6\text{H}_5)_3$, whereas $\text{HSi}(\text{C}_2\text{H}_5)_3$ is not effective. This reactivity pattern parallels the expected order of Rh-Si bond strength⁹.

Heating **2a** with $(\text{H}_3\text{CO})_3\text{SiSi}(\text{OCH}_3)_3$ at 100 °C results in high-yield detachment of a CH_2 group from **2a** and its insertion into the Si-Si bond, yielding $(\text{H}_3\text{CO})_3\text{SiCH}_2\text{Si}(\text{OCH}_3)_3$ (**5**). The identity of this compound is unequivocal, based on ^1H , ^{13}C and

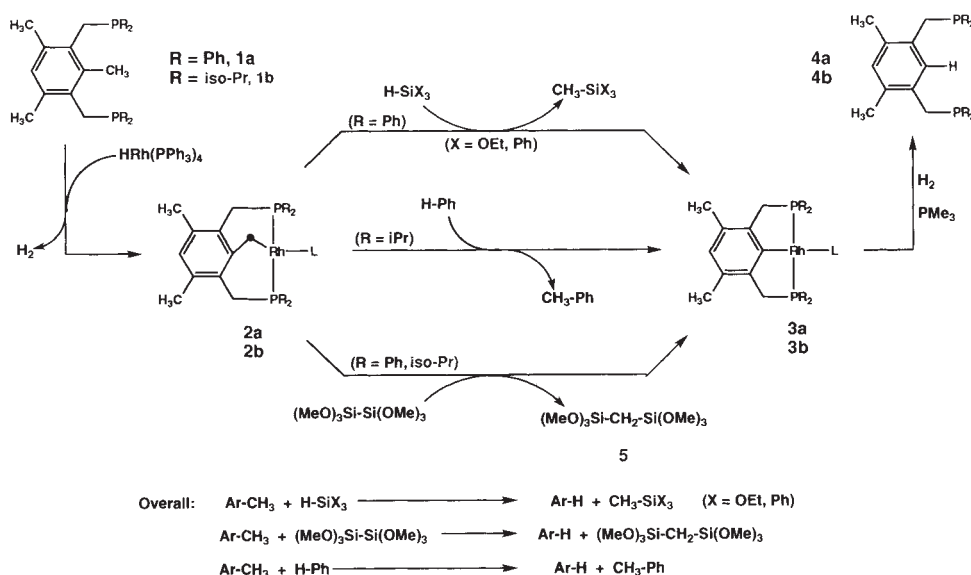
^{29}Si NMR, ^{13}C DEPT (distortionless enhancement by polarization transfer) NMR and mass spectrometry (see Supplementary Information). Complex **3a** is formed quantitatively, leading to the arene **4a** (Fig. 2). When the deuterium-labelled complex **2c** is used, $(\text{H}_3\text{CO})_3\text{SiCD}_2\text{Si}(\text{OCH}_3)_3$ is selectively formed, confirming unambiguously the origin of the methylene group (Fig. 3). Similar reactivity is observed with **1b**, leading to **5** and **4b**. We are unaware of other approaches for the direct transformation of R_3SiSiR_3 into $\text{R}_3\text{SiCH}_2\text{SiR}_3$. The reported synthesis of **5** involves pyrolysis of CH_3SiCl_3 at 700 °C followed by alcoholysis¹⁰. Interestingly, complex **2a** is available from **3a** by reaction with CH_3I and a base (M.G. and D.M., unpublished results). This, in effect, makes CH_3I a source of CH_2 for incorporation into Si-Si (as well as into other bonds).

Significantly, when this approach is combined with C-H activation, transfer of CH_2 from one hydrocarbon to another results. Thus, an overall transfer of a CH_2 group from compound **1b** to benzene (yielding toluene and **4b**) was accomplished by forming complex **2b** and heating it with benzene to 110 °C, yielding complex **3b**, from which compound **4b** was released. In one remarkable step, **2b**→**3b**, selective C-C cleavage, C-H cleavage and C-C bond formation are involved, all under mild conditions in solution.

A plausible mechanism for these conceptually new transformations is presented in Fig. 4. The first step, oxidative addition of C-H and Si-H bonds to a low-valent centre, has many precedents^{3,6,9,11}. Although the oxidative addition products were not directly observed here, the existence of equilibria **2**⇌**6** is strongly supported by the equilibrium reactions between the deuterated complexes, **2c**, **2d** and $(\text{H}_3\text{C}_2\text{O})_3\text{SiH}$, as well as C_6H_6 (Fig. 3). Formation of $(\text{H}_3\text{C}_2\text{O})_3\text{SiD}$ and $\text{C}_6\text{H}_5\text{D}$ is unambiguous, based on ^1H and ^2H NMR. The observed order of reactivity of silanes, mentioned earlier, reflects the fact that the oxidative addition is more favourable when stronger Rh-Si bonds are formed.

Reductive elimination of C-H from **6**, followed by metal insertion into Ar-C (Ar, aryl; ref. 7) would lead to intermediate **7**. Reductive elimination of C-Si or C-C would generate the observed products. A mechanism involving C-Si rather than C-H reductive elimination from **6** is also possible, especially in light of our recent finding that C-Si and C-H reductive elimination reactions can proceed at competitive rates¹².

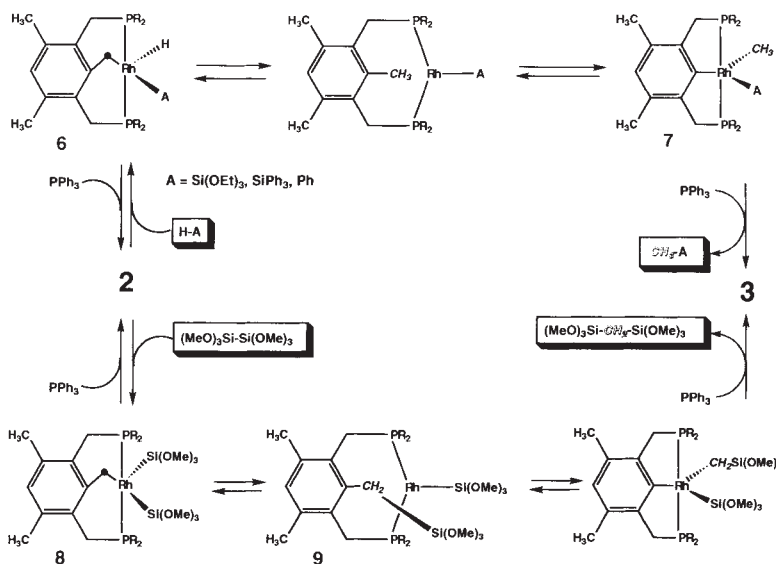
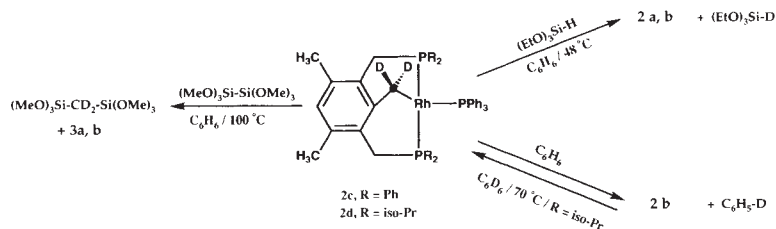
FIG. 2 Experiments showing selective transfer of a CH_2 group from **1a**, **b** and its selective incorporation into Si-H, C-H and Si-Si bonds (Ar, aryl). METHODS. A suspension of 600 mg of $\text{HRh}(\text{PPh}_3)_4$ and 268 mg of **1a** in 100 ml of tetrahydrofuran was stirred at 25 °C for 12 h under N_2 ; the resulting solution was concentrated under vacuum to a volume of 5 ml. Addition of 30 ml of pentane caused precipitation of an orange-yellow powder (**2a**) which was washed with pentane and vacuum-dried to yield 440 mg of **2a**. Complex **2b** was prepared similarly. In a typical experiment, a solution of 200 mg of **2a** and 240 mg of $\text{HSi}(\text{OEt})_3$ in 6 ml of C_6D_6 was heated under N_2 at 100 °C for 24 h. The resulting solution was analysed by ^1H , ^{31}P , ^{13}C , ^{13}C DEPT and ^{29}Si NMR, showing quantitative formation of **3a** and $\text{CH}_3\text{Si}(\text{OEt})_3$. The volatile components of the reaction were separated under vacuum and were analysed quantitatively by GC-MS and NMR, confirming formation of $\text{CH}_3\text{Si}(\text{OEt})_3$. Reaction of $(\text{MeO})_3\text{SiSi}(\text{OMe})_3$ was conducted similarly,



except that 1 ml of this compound was used. In reaction of **2b** with benzene, heating at 110 °C for 36 h was required.

FIG. 3 Deuterium-labelling experiments.

METHODS. Complexes **2c** and **2d** were obtained by reaction of C_6D_6 solutions of **2a** and **2b** (respectively) with D_2 (20 p.s.i.) at 25 °C for 6 h. Complete H/D exchange was verified by 1H and 2H NMR. Reactions of **2c**, **2d** with $HSi(OEt)_3$, $(MeO)_3SiSi(OMe)_3$ and C_6H_6 were performed under conditions similar to those described in Fig. 2, at the temperatures specified in Fig. 3. The products were analysed by 1H , 2H , ^{31}P NMR and GC-MS.

FIG. 4 Plausible mechanism for the CH_2 transfer reactions. (Compounds **2** and **3** defined in Figs 2, 3.)

An analogous mechanism accounts for CH_2 insertion into the Si-Si bond. Oxidative addition of Si-Si, although relatively uncommon, has been reported¹³. C-Si reductive elimination of the postulated compound **8** would generate complex **9**. C-C insertion and C-Si bond formation account for the observed products. It is interesting to note that a Rh-SiR₃ moiety is capable of insertion into a C-C bond.

Also noteworthy is that these CH_2 transfer reactions are likely to be thermodynamically favourable. Indeed, hydrosilylation of $C_6H_5-CH_3$ (bond dissociation energy, $101.8 \pm 2 \text{ kcal mol}^{-1}$)¹⁴ with $H-Si(OC_2H_5)_3$ ($\sim 90 \text{ kcal mol}^{-1}$)¹⁵ to yield C_6H_5-H ($110.9 \pm 2 \text{ kcal mol}^{-1}$)¹⁴ and $CH_3-Si(OC_2H_5)_3$ ($\sim 91 \text{ kcal mol}^{-1}$)¹⁵ is expected to be exothermic by $\sim 10 \text{ kcal mol}^{-1}$. Likewise, the process of CH_2 transfer from $C_6H_5CH_3$ to $(H_3CO)_3SiSi(OCH_3)_3$ should also be exothermic ($\Delta H^\circ \approx 14 \text{ kcal mol}^{-1}$; bond dissociation energies of Si-Si, ~ 85 ; Si- CH_2 $\sim 90 \text{ kcal mol}^{-1}$ (ref. 15)). Transfer of CH_2 between two different arenes may be approximately thermoneutral.

For comparison, the industrially important rupture of C-C bonds in saturated aromatic and aliphatic hydrocarbons normally occurs under extreme conditions, such as high temperature¹⁶ or in super-acidic media¹⁷, when the reactions are non-selective and cannot tolerate many functional groups. Photochemical cyclo-elimination of carbenes from compounds of the very strained cyclopropyl ring has been reported¹⁸. β -alkyl cleavage in highly Lewis-acidic complexes is known^{19,20}, and has recently led to a novel polymerization process involving a strained hydrocarbon²¹.

Although the full potential of the approach reported here awaits exploration, it is already clear that CH_2 -group transfer is a viable concept. The scope of this reaction can be quite broad with regard to the CH_2 acceptor, as already demonstrated for typical molecules containing Si-H, Si-Si and C-H bonds. Regarding the CH_2 hydrocarbon donor, coordination assistance

is so far required in order to direct the metal at the C-C bond to be cleaved. Other substrates that contain an appropriately oriented metal binding site are likely to show similar reactivity. Taking into consideration the range of groups capable of metal coordination, we believe that the concept of methylene transfer may be of broad applicability. □

Received 14 March; accepted 26 May 1994.

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SUPPLEMENTARY INFORMATION. Data on product characterization by mass spectrometry and 1H , ^{31}P , ^{29}Si and ^{13}C NMR are available. Requests should be addressed to Mary Sheehan at the London editorial office of *Nature*.

ACKNOWLEDGEMENTS. This research was supported by MINERVA Foundation, Munich, Germany, and by the Fufeld Foundation. We thank Hoechst AG for a gift of chemicals.

Electrochemical enhancement of a catalytic reaction in aqueous solution

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THE rates of many heterogeneous catalytic reactions between gaseous adsorbates on metal surfaces supported on solid electrolytes can be increased by applying a potential to the metal¹⁻⁸. This phenomenon, which has been reported previously at temperatures of 250–750 °C, has been shown⁹ to be due to the electrochemically induced spillover of ions from the support onto the catalyst surface; the ions then act as promoters for the catalytic reaction. Here we report that a similar effect can be observed in aqueous solution at ambient temperatures. We studied the oxidation of H₂ on a platinum/graphite electrode immersed in aqueous KOH. Application of a positive potential of 1–2 V to the Pt electrode increased the rate of H₂ oxidation by up to 500%. We deduce that hydroxide ions are acting as promoters, and find that each ion supplied to the catalyst causes the oxidation of up to 20 hydrogen atoms. This kind of rate enhancement for heterogeneous catalytic reactions in solution may be of considerable technological value, for example in the electrochemical treatment of toxic organics¹⁰ or the generation of useful industrial chemicals⁴.

The anodic oxidation of H₂ on Pt and other metal electrodes has been studied thoroughly¹¹⁻¹⁴, but not under the conditions considered here, in which oxygen and hydrogen are fed simultaneously over the same electrode. Aqueous electrolyte electrochemical processes, such as the anodic oxidation of H₂ in fuel cells^{12,13} or of toxic organic molecules in waste-treatment electrochemical units¹⁰, operate with a faradaic efficiency Λ ($=r/(I/2F)$) of at most 100% (refs 10, 12, 13). Here r is the rate of fuel oxidation at the anode expressed in mol-O s⁻¹, I is the cell current and F is Faraday's constant. When oxygen is not supplied to the anode along with H₂, as in all previous electrochemical studies, then the anodic electrode can act as an electrocatalyst only for net charge-transfer reactions, for example anodic H₂ oxidation, the rate of which is limited to $I/2F$ by Faraday's law. Consequently Λ is limited to values of at most 100%.

The catalyst-electrode was finely dispersed Pt supported on graphite (Stonehart Associates). The Pt loading of the carbon support was 20 wt%, with a Pt crystallite surface of 3.5×10^4 m² per mol-Pt. The total Pt surface area of the catalyst-electrode was 1,200 cm², that is 1.8×10^{18} Pt atoms or 3.0×10^{-6} mol Pt. The electrode was deposited on a Teflon frit immersed in a 0.1 M KOH aqueous solution placed in a thermostatted glass vessel of volume 100 cm³. The catalyst-electrode potential E , corrected for the uncompensated ohmic drop^{3,4}, was continuously monitored with respect to a reference H₂ electrode (r.h.e.) immersed in the same solution. The Pt counter-electrode was situated in a separate compartment, electrolytically connected by a cationic Flemion membrane. In this way the rates of H₂ and O₂ consumption on the working catalyst-electrode could be accurately measured without any interference from the gases produced or consumed at the counter-electrode. Results reported here were obtained at 25 °C. Mixtures of high purity H₂ and O₂ diluted in He were bubbled continuously through the Teflon frit at rates of 200–600 cm³ min⁻¹ at STP. On-line gas chromatography and mass spectrometry was used to monitor the rates r_{H_2} (mol-H₂ s⁻¹) and r_O ($\frac{1}{2}$ mol-O₂ s⁻¹) of H₂ and O₂ consumption, respectively. Water vapour in the product line was trapped

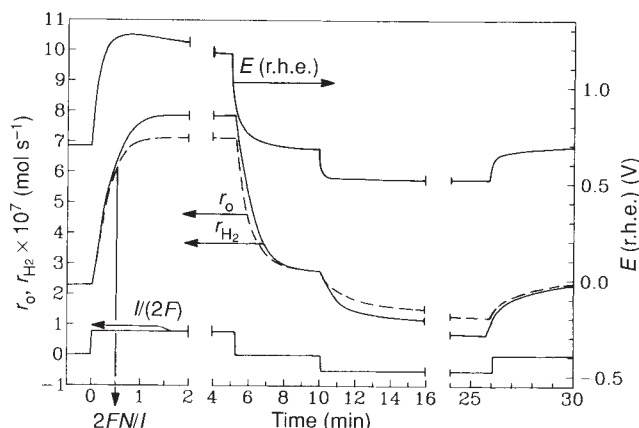


FIG. 1 Transient effect of applied positive and negative currents ($I = +15$ and -10 mA) on the rates of consumption of hydrogen (r_{H_2}) and oxygen (r_O); $p_{H_2} = 0.75$ kPa, $p_{O_2} = 1.06$ kPa; gas flowrate $Q = 280$ cm³ min⁻¹ at STP.

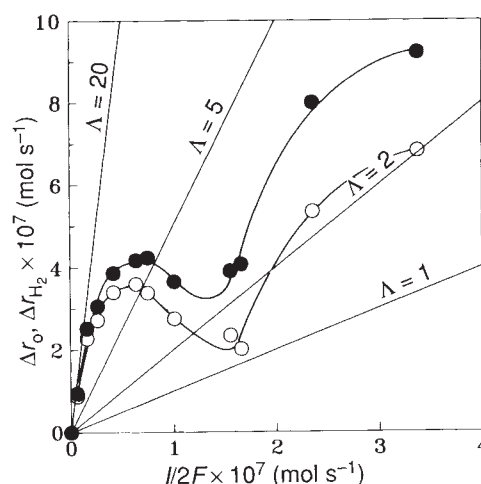
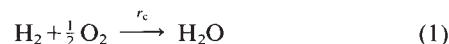


FIG. 2 Steady-state effect of applied positive (anodic) current (I) on the increase in the rates of hydrogen (●) and oxygen (○) consumption. p_{H_2} and p_{O_2} are 0.8 and 1.25 kPa, respectively. Straight lines are lines of constant faradaic efficiency; $r_{H_2}^0$ ($=r_O^0=r_C^0$) = 2.38×10^{-7} mol s⁻¹ is the open-circuit catalytic rate; $Q = 540$ cm³ min⁻¹ at STP.

continuously in a silica-gel column located before the gas analysis unit. The gas flow rate was chosen such that the conversion of H₂ and O₂ was maintained under all conditions below 40%. The absence of diffusional limitations was verified by varying the total flowrate between 100 and 600 cm³ min⁻¹ at STP and observing no significant change in the rates of consumption. No problems with deterioration of catalyst or support were encountered over prolonged periods (~200 h) of operation at potentials below 1.5 V. Substitution of the 0.1 M KOH solution with a 0.1 M LiOH solution led to qualitatively similar results.

Hydrogen and oxygen are consumed on the Pt surface at a rate r_c by the catalytic reaction:



Under open-circuit conditions the catalyst potential E (r.h.e.) takes values of the order 0.4–0.85 V, that is -0.35 to $+0.1$ V on the normal hydrogen electrode scale (n.h.e.), depending on the hydrogen to oxygen ratio.

When a positive current I is applied between the catalyst-electrode and the Pt counter-electrode, then the catalyst potential

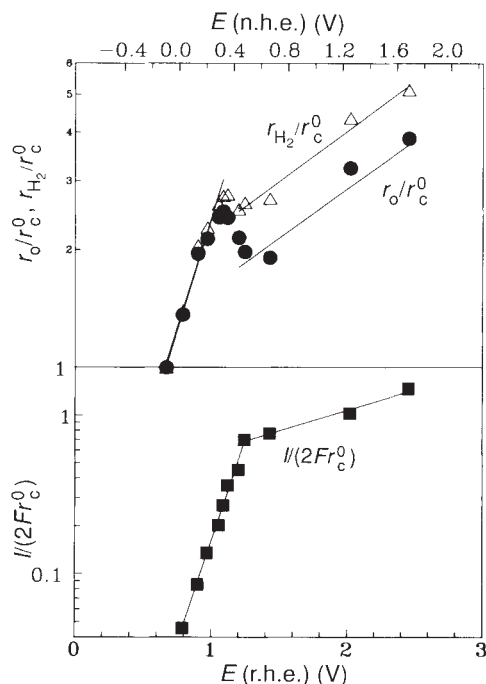
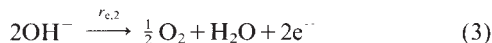
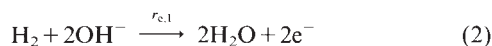


FIG. 3 Steady-state effect of ohmic-drop-free catalyst potential on current I (■) (bottom), and on the rates of hydrogen (Δ) and oxygen ($\bullet$) consumption (top); r_c^0 ($=r_O^0=r_H2^0$) $=2.38 \times 10^{-7} \text{ mol s}^{-1}$ is the open circuit catalytic rate; $Q=540 \text{ cm}^3 \text{ min}^{-1}$ at STP; p_{H_2} and p_{O_2} as in Fig. 2.

E changes to more positive values (Fig. 1) and the following electrochemical (net charge-transfer) reactions take place at the Pt catalyst-electrode surface^{11, 14}:



where the forward reaction (3), that is $r_{c,2} > 0$, takes place when E (r.h.e.) is above the oxygen reduction potential (1.23 V) and the reverse reaction, that is $r_{c,2} < 0$, occurs otherwise. It follows that in general $r_{H_2} = r_c + r_{c,1}$, $r_O = r_c - r_{c,2}$, $I/2F = r_{c,1} + r_{c,2}$ and thus

$$r_{H_2} - r_O = I/2F \quad (4)$$

Consequently if r_c were to remain constant, application of a positive current, I , would cause a decrease in r_O ($\Delta r_O \leq 0$) and an increase in r_{H_2} ($\Delta r_{H_2} \leq I/2F$). Surprisingly, as shown in Fig. 1, r_O increases by 310% relative to its open-circuit value r_c^0 ($2.3 \times 10^{-7} \text{ mol-O s}^{-1}$) whereas the increase in r_{H_2} ($\Delta r_{H_2} = 5.6 \times 10^{-7} \text{ mol-H}_2 \text{ s}^{-1}$) is 344% relative to the open-circuit value $r_{H_2}^0 = r_O^0 = r_c^0$ and 720% higher than $I/2F$. The faradaic efficiency Λ (refs 1-7), defined as $\Delta r_{H_2}/(I/2F)$, is therefore 7.2 (720%) for the galvanostatic experiment of Fig. 1. The non-faradaic behaviour ($\Lambda > 1$) is due to the electrochemical activation of the catalytic reaction (1), the rate (r_c) of which increases by between 310 and 344%. Fig. 1 also shows that:

- (1) The rate relaxation time constant τ on constant current application is $\sim 2FN/I$, where N (mol Pt) is the Pt catalyst-electrode surface area.
- (2) The effect is reversible, that is r_{H_2} , r_O and catalyst potential E all return to their open-circuit values on current interruption.
- (3) Negative currents also cause a non-faradaic decrease in r_{H_2} and r_O .
- (4) At steady-state, the difference $r_{H_2} - r_O$ always equals $I/2F$ according to equation (4).

Figure 2 shows the steady-state effect of positive current on

r_{H_2} and r_O . The faradaic efficiency Λ exceeds 20 (2,000%) for low currents. The corresponding effect of catalyst potential E on r_{H_2} and r_O is shown on Fig. 3, together with the dependence of I on E .

The break in the plot I against E coincides with the observed inflection in r_{H_2} and r_O , and corresponds to the onset of Pt oxide formation^{11, 13}. As shown in Fig. 3, the predominantly catalytic rates r_{H_2} and r_O depend exponentially on catalyst potential E , as in studies on solid electrolytes^{1, 7}, with slopes comparable to the Tafel^{4, 12} ($\log I$ against E) slopes seen here. This explains why⁴ the observed magnitude of the faradaic efficiency Λ ($\sim 2-20$) is in good agreement with the parameter $2F r_c^0/I_0$, where I_0 is the exchange current¹⁻⁴, which is known to predict the expected magnitude of Λ in solid-electrolyte studies¹⁻⁷.

Figure 4 shows the effect of varying p_{O_2} , p_{H_2} and catalyst potential on the rate of H_2 oxidation which obeys Langmuir-Hinshelwood-type kinetics. Increasing E causes both a pronounced increase in r_{H_2} , and a shift of the rate maximum to higher p_{O_2} and lower p_{H_2} values. This shift indicates a weakening in the Pt=O chemisorptive bond and a strengthening in the Pt-H bond, both consistent with the expected effect of increased potential and work function^{3, 4, 13} on the binding strength of the electron acceptor (oxygen) and electron donor (hydrogen) adsorbates. These considerations can also account for the appearance of the local catalytic rate maximum with respect to potential to the left of the break in the $I-E$ curve (Fig. 3).

Thus, as in the case of solid-electrolyte electrochemistry^{3, 4}, the observed phenomenon appears to be due to the effect of the changing catalyst potential and work function^{3, 13} on the binding strength of the adsorbates (that is Pt-H and Pt=O) that participate in the catalytic reaction^{3, 4, 11, 13, 15}. It does not seem to be due to surface area effects, such as movement of the three-phase (metal-gas-solution) boundary due to the changing potential.

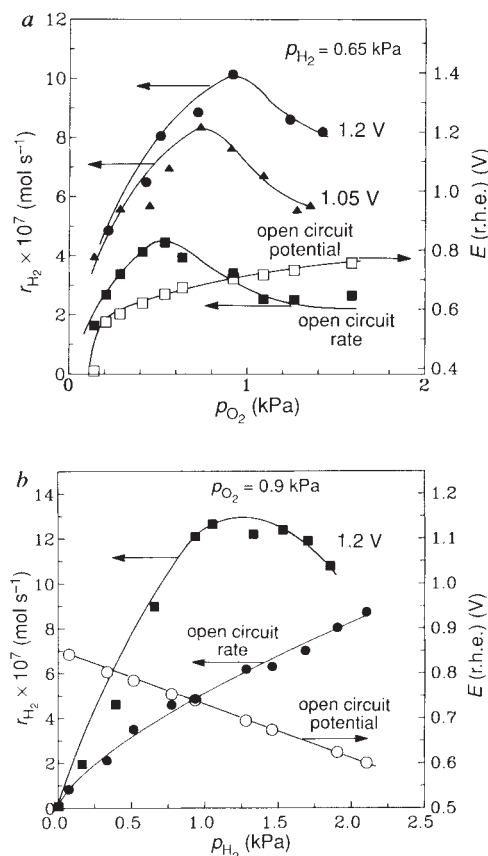


FIG. 4 Effect of electrode-catalyst potential and oxygen (a) and hydrogen (b) partial pressure on the rate of hydrogen oxidation in 0.1 M KOH (a) and 0.1 M LiOH (b); $Q=500 \text{ cm}^3 \text{ min}^{-1}$ at STP.

Changes in the binding strength of adsorbates with electrode potential have been inferred from previous cyclic voltammetric¹¹, surface spectroscopic^{13,15} and oxide reduction studies^{11,13}. The present results establish, however, that the electrode potential affects not only adsorption energetics but also the kinetics of catalytic reactions taking place on electrode surfaces. The effect is pronounced, leading to faradaic efficiencies well in excess of 100% and up to 2,000%.

A full understanding of the origin of the effects reported here will have to await a more complete understanding of the changes in binding strength of adsorbates and reaction intermediates, including the transition state¹³, with changing potential, work function and local field at the interface^{15,16}. *In situ* spectroscopic¹⁵ and theoretical¹⁶ studies are needed to further elucidate these issues. □

Received 9 December 1993; accepted 12 May 1994.

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ACKNOWLEDGEMENTS. This work was supported by the CEC JOULE Programme.

Stimulation of methane emission by carbon dioxide enrichment of marsh vegetation

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THERE is substantial evidence that many plants respond to increased concentrations of atmospheric carbon dioxide by increasing their productivity^{1–4}. This observation has led to the suggestion that, by taking up CO₂, the terrestrial biosphere might mitigate the potential greenhouse warming associated with anthropogenic CO₂ emissions⁵. Whiting and Chanton⁶ have found, however, that for wetlands of varying productivity around the world, higher net primary production is associated with higher emissions of methane—another important greenhouse gas. Here we present measurements of methane emissions from a marsh that has been exposed to twice the present ambient concentration of atmospheric CO₂. We find that over a one-week period, the CO₂-enriched sites had significantly higher emissions of methane than the control sites. Our results suggest that future increases in atmospheric CO₂ concentration may lead to significant increases in methane emissions from wetlands.

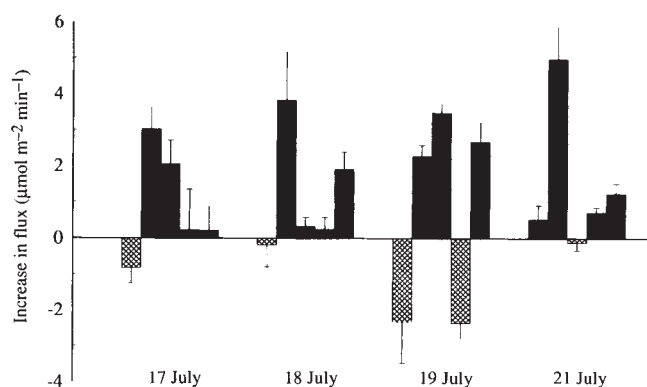


FIG. 1 Difference in methane emission from the sedge *Scirpus olneyi* exposed to elevated CO₂ versus control sites on four different days.

METHODS. Data are plotted as the difference (with standard error) in CH₄ emission between elevated CO₂ chambers and control chambers for five pairs of chambers on four nights. Emissions from plots were variable from day to day and from chamber to chamber. Probit plot indicates that data are log-normally distributed, as Aselman and Crutzen²³ suggest is typical for CH₄ emissions. Two-way analysis of variance shows no block effect or day effect, but does suggest a strongly significant effect of enhanced CO₂ on CH₄ emission. Emissions of methane are significantly higher in CO₂-enriched chambers ($F = 0.012$) than ambient chambers. Under normal conditions, air is pumped through the chambers to achieve a turnover time of 15 s to minimize the effects of enclosure on the vegetation. Although measuring the change in CO₂ content between inlet and outlet air permits measurement of CO₂ flux, the flux of CH₄ from the marsh is insufficient to allow estimation in the same way. Rates of CH₄ emission for each chamber were determined from the rate of increase in CH₄ concentration inside the chambers after turning off the main air flow and sealing the chamber. Samples were drawn at 90-s intervals for timespans of 4.5 to 6 min (4 or 5 data points each run) during the first half of the night during four nights in July 1991. The CH₄ concentration of the samples was determined in replicate runs for each sample by flame ionization gas chromatography. Solid bars, occasions when CH₄ emission was higher in the CO₂-enriched chamber than in its ambient control; hatched bars, occasions when CH₄ emission was higher in the ambient chamber. Rates of CH₄ emission ranged from 0.23 to 4.78 $\mu\text{mol m}^{-2} \text{min}^{-1}$ (mean = 1.37) in control chambers, from 0.41 to 5.96 $\mu\text{mol m}^{-2} \text{min}^{-1}$ (mean = 2.46) in CO₂-enriched chambers.

Perhaps the best documented of the many environmental changes discussed within the framework of global climate change is the rise in atmospheric CO₂. Long-term records demonstrate a steady rise in atmospheric CO₂ since the pre-industrial era, with an accelerated rate of rise in recent decades⁷. Attention has focused on this rise because the infrared-absorbing properties of CO₂ may lead to a warming of the global atmosphere⁸. Methane is another major greenhouse gas which is increasing in atmospheric concentration: CH₄ is present at only about 1.7 parts per million by volume (p.p.m.v.) in the atmosphere, but it is more than 20 times more efficient at infrared absorption than CO₂ on a mole-per-mole basis⁹.

Approximately 40% of the methane entering the atmosphere originates from wetlands and rice paddies¹⁰. Methane from these sources is a product of the anaerobic decomposition of organic matter, most of which is derived from the roots and rhizomes of plants inhabiting the sediment^{11,12}. Although wetlands may not represent an important sink for CO₂ on a global scale, changes in carbon cycling in these systems may lead to significant changes in the concentrations of other greenhouse gases. For instance, altering the relative contributions of aerobic and anaerobic processes to decomposition will influence the extent of CH₄ formation.

Although it has been recognized that changing climate may affect CH₄ emission^{13–15}, the potential feedback between CO₂ and CH₄ has not been investigated in detail before this study. We measured CH₄ emission in a sedge community where a sustained

increase in carbon storage has been observed in response to experimental CO₂ elevation. Our measurements suggest that the uptake of CO₂ by wetland vegetation may actually amplify the greenhouse effect of elevated CO₂ by increasing the flux of CH₄ to the atmosphere.

Most plant species fix CO₂ by the C3 pathway; the biochemistry of C3 plants suggests that most of these plants will increase their photosynthesis in response to increased atmospheric CO₂ (refs 1, 2). Most evidence suggests that this stimulation of photosynthesis leads to increasing plant growth^{3,4}. A number of field studies with native plant species show that much of this increasing growth occurs below ground¹⁶⁻¹⁸. The Smithsonian CO₂ enrichment experiment is a block design in five triads: a CO₂-enriched (twice normal ambient, ~690 p.p.m.v.) chamber, a control (normal ambient) chamber, and a companion plot without a chamber. Open-top chambers (0.8 m diameter, 1.5 m tall) are used to elevate the CO₂ content of the atmosphere around the leaves of the vegetation, with a residence time for flowthrough air of 15 s (refs 19, 20). Previous results in this marsh have shown that doubling the concentration of ambient CO₂ has resulted in an increase in photosynthesis²¹ and carbon storage²² by the sedge *Scirpus olneyi* (a C3 plant) (Table 1; B.G.D., unpublished data): this increase in net ecosystem CO₂ assimilation (NEC) has persisted since the treatment began 7 years ago. There was year-to-year variability in each of these measurements, but there were no trends to indicate that the effects of elevated CO₂ have decreased since the beginning of the CO₂-enrichment experiment (B.G.D., unpublished data). The average augmentation in annual NEC from 1988 to 1992 is 43%.

The sedge is typical of native perennial plants in that it stores most of its organic matter in roots and rhizomes buried in the sediment. Below-ground biomass represents a source of organic carbon for decomposition, and because wetland sediments are typically anoxic, the pathway for decomposition of buried material is dominated by anaerobic processes. After CO₂, CH₄ is the main carbon-bearing gas emitted from these systems.

We measured the emission of CH₄ by quantifying the rate of increase in CH₄ concentration in temporarily closed chambers^{19,20}. Methane emission from the CO₂-enriched sites was nearly 80% higher than in control sites (Fig. 1). Control chambers exposed to normal ambient CO₂ released an average of 1.37 (±0.28 standard error) µmol m⁻² min⁻¹, whereas emission from CO₂-enriched chambers averaged 2.46 (±0.34 standard error) µmol m⁻² min⁻¹. Extrapolating these rates to a daily flux, the rates are 1.97 and 3.54 mmol m⁻² d⁻¹, respectively. These fluxes are substantial, especially given that the sedge inhabits a brackish saltmarsh^{23,25}. Measurements were made within 3 weeks of peak plant biomass, at the time when most studies suggest CH₄ emissions would be at their seasonal highest^{23,26}.

The importance of increasing carbon storage and its consequences for CH₄ emission can be evaluated only through long-term study. Just as photosynthesis exhibits a strong annual cycle, so do decomposition and CH₄ emission¹¹. In wetland systems, with their extensive allocation of organic matter to below-ground organs, the flux of CH₄ will tend to be higher with increased production⁶. The rate of CH₄ emission has also been linked to the input of organic carbon in lake sediments²⁷. The processes controlling the production and emission of CH₄ from wetlands are many and variable. The nature and quantity of the organic matter are important, but we do not know what fraction of CH₄ produced is derived from the decomposition of old organic matter, and what fraction is derived from leakage of relatively recently formed organic matter from roots in any wetland. Recent work correlating CH₄ emission and CO₂ fixation in short-term experiments implies that CH₄ emission may be fairly closely coupled to net ecosystem production⁶. Another variable in the plant-sediment system is the role played by the plants themselves in transporting methane from the sediment. Higher plants inhabiting wetlands typically have a well-developed system of internal gas spaces. These spaces have the bio-

logical function of transporting O₂ from the atmosphere to roots buried in anaerobic sediments, but they also accelerate the CH₄ transport from the sediment to the atmosphere^{28,29}. Increasing the root biomass may increase the surface area for flux into gas spaces within the roots, thereby enhancing the CH₄ stripping from the sediment. It is also possible that in some systems increased biomass could have the opposite effect. Increased root biomass may promote methane oxidation in the rhizosphere or may decrease methanogenesis by transporting additional O₂ into the sediment, although these effects are not evident in this study. Our research focused on the emissions only, which showed an increase in response to elevated atmospheric CO₂ concentration.

Wetlands play an important role in greenhouse-gas budgets, and production by wetland vegetation may therefore act as an amplifier for production of greenhouse gases. Although increases in the biological uptake of CO₂ may moderate the rise of atmospheric CO₂ concentration, changes in the pathways for decomposing the additional organic matter may exacerbate the greenhouse effect through increased CH₄ emission. Even in cases where biomass storage may not be a major response to elevated CO₂, there are strong indications of increased root activity¹⁶ which would undoubtedly have consequences for trace gas production. Diaz *et al.*³⁰ investigated the response of vegetation to elevated CO₂ in growth chambers, and concluded, from increased carbon/nitrogen (C/N) ratios in the leaves, that the effect of CO₂ enrichment can only be temporary due to nitrogen limitation. Contrary to that conclusion, the Smithsonian study³¹ suggests that C/N ratio should not be used as an indicator of nitrogen limitation, as elevated C/N ratios have accompanied the sustained augmentation of plant growth. It appears that the requirement for protein, specifically the primary carboxylating enzyme Rubisco (which represents ~50% of soluble, non-structural leaf protein in many plant species²), is diminished in plants grown in CO₂-enriched atmospheres. In any case (as discussed earlier), there is considerable evidence that the increase in net ecosystem CO₂ assimilation has persisted at the present study site throughout the 7 years of treatment. This, taken together with the idea that wetlands with high productivity have more methane emissions⁶, implies that the enhancement of CH₄ emissions that we report have occurred at the treated site throughout the 7 years of treatment.

The increase in CH₄ emission that we have observed may be of sufficient magnitude to influence atmospheric chemistry. The mechanism we describe here suggests that CO₂ has a positive

TABLE 1 Effect of CO₂ enrichment on the growth of sedge during 1991

	Enhanced CO ₂	Ambient	Increase (%)
Daily NEC (mol-C m ⁻²)			
21 June	1.56	1.13	38
8 August	1.02	0.82	24
Total NEC (mol m ⁻²)			
May–Nov	135	108	26
New root biomass (mol-C m ⁻² yr ⁻¹)	112	63	76
Total shoot number (m ⁻²)	1,810	1,520	19

Net ecosystem CO₂ assimilation (NEC) was estimated by monitoring the change in CO₂ content between inlet and outlet for each chamber (ref. 20). Gas exchange data are means of five replicate chambers at each CO₂ treatment; values are different at the *P*=0.05 level by the Student's *t*-test. Examples of daily and seasonal NEC data are included to demonstrate the consistency of the increase in CO₂ fixation. The effect of elevated CO₂ on root and rhizome dynamics was estimated as the biomass of new roots and rhizomes produced during the 1991 growing season in re-growth cores removed in December (ref. 18). This measurement is not exactly equivalent to below-ground production, but it indicates a substantial increase in new root production under CO₂ treatment. Total shoot number, an expression of above-ground production, was estimated by the number of shoots in each chamber at the peak of the growing season.

indirect effect on greenhouse warming. Thorough evaluation of this biotic feedback will require more experiments with other vegetation in other wetlands, and at present there are no other wetland studies suitable for such an investigation of CH₄ emission. The response of rice will be particularly important in this regard, as it is a C3 plant and likely to exhibit increased production in response to increased CO₂. Rice is generally well fertilized and is cultivated in warm environments: both factors tend to enhance the direct effect of CO₂ on photosynthesis² and growth.

Our observations may be significant in the context of the observed annual 1–1.5% rise (~ 10 p.p.b. yr⁻¹) in the atmospheric CH₄ concentration. Several processes have been previously identified which undoubtedly contribute to the rise, from changes in the sources of CH₄ emission (rice cultivation and ruminant population), to changes in the rate of atmospheric oxidation of CH₄ (ref. 10). Although it is unlikely to be a major force behind the rise in CH₄, a CO₂-driven feedback may also be a factor. If the historical rise in atmospheric CO₂ has already resulted in increased plant production in wetlands, we would expect that a concomitant rise in CH₄ emission has also occurred. The contribution of wetlands to the global CH₄ budget is substantial, and small changes in this source can have a significant effect.

Biological feedback in response to global change is emerging as an important area of study. The responses of vegetation are central to understanding the possibilities for feedback in the biosphere, because vegetation supplies virtually all the organic matter for the biosphere. Vegetation influences, both directly and indirectly, the emission of a variety of gases that are important in atmospheric chemistry³². Changes in the cycling of carbon will inevitably lead to changes in other elemental cycles, and will undoubtedly have unanticipated consequences for greenhouse-gas dynamics. □

Received 2 March 1993; accepted 18 May 1994.

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ACKNOWLEDGEMENTS. We thank R. W. Hill for assistance in statistical analysis, and G. Peresta, A. Richardson, P. Utley and W. Pockman for assistance in the field. This work was supported by NASA, the Smithsonian Institution, and the US DOE and NSF.

Importance of vegetation in removing polycyclic aromatic hydrocarbons from the atmosphere

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ANTHROPOGENIC semi-volatile organic compounds such as polycyclic aromatic hydrocarbons (PAHs) are highly lipophilic (which makes them likely to accumulate in animal tissue), and some are carcinogenic or mutagenic¹. Although such compounds are known to accumulate in vegetation^{2–5}, little is known about the quantitative role played by vegetation in removing them from the atmosphere. We have developed a mass-balance model for PAHs for the northeast of the United States, based on measurements of PAHs in soil and vegetation from Bloomington, Indiana, and published values for PAH concentrations and fluxes in air, water, sediments and soils. Our model shows that $44 \pm 18\%$ of the PAHs emitted into the atmosphere from sources in this region are removed by vegetation. Although the equilibrium between the atmosphere and vegetation depends on ambient temperature⁶, we believe that most of the PAHs absorbed by vegetation at the end of the growing season are incorporated into the soil^{7,8} and permanently removed from the atmosphere.

Atmospheric semi-volatile organic compounds include anthropogenic compounds such as PAHs, polychlorinated biphenyls and organochlorine pesticides. Their atmospheric concentrations are generally 0.001 – 10 ng m⁻³ (10^4 – 10^8 molecules per cm³), and they partition between the atmospheric gas-phase and particle-phase. Some semi-volatile organic compounds, such as PAHs, are products of incomplete combustion, whereas others, such as polychlorinated biphenyls, were produced by the chemical industry. Semi-volatile organic compounds are transported globally and are found in the atmosphere, water, sediment, soil and biota, even at remote locations. The worldwide occurrence and lack of controlled use of these toxic compounds in many countries suggests that global budgets should be developed to assess human exposure, and to determine the routes by which these compounds are removed from the atmosphere.

We have determined the magnitude of the role of vegetation in removing these compounds from the atmosphere by developing a regional mass-balance model using PAHs as a surrogate for other semi-volatile organic compounds. PAHs are excellent surrogates because of their wide range of vapour pressures (some atmospheric PAHs exist exclusively in the gas-phase and others in the particle-phase) and lipophilicities. The mass-balance model was calculated for the northeastern region of the United States; the area is bordered by North and South Dakota, Nebraska, Kansas, Missouri, Kentucky and Virginia, inclusive. The area of this region is $\sim 3.3 \times 10^6$ km²; $\sim 10\%$ of this area is the Great Lakes, $\sim 70\%$ is covered by crops, grasslands and urban vegetation, and $\sim 20\%$ is forested. About 52% of the population of the United States lives in this area.

We measured the concentrations of ten PAHs (phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, triphenylene, benzo[e]pyrene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene and benzo[ghi]perylene) in 147 vegetation and soil samples at a suburban site in Bloomington, Indiana⁶. Sugar maple (*Acer saccharum*) leaves and seeds were collected to represent broad-flat plant surfaces, white pine (*Pinus strobus*)

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needles (both new growth and the previous year's needles) were collected to represent narrow-round plant surfaces, and sugar maple and white pine bark were collected to represent rough plant surfaces. These different plant tissue types also represent varying degrees of lipid content; white pine bark had the highest average lipid content (170 mg-lipid per g dry wt), followed by sugar maple bark, white pine needles, sugar maple leaves and sugar maple seeds, with 33, 23, 16 and 6 mg-lipid per g dry weight, respectively. Collection of leaf, seed, and needle samples began in April 1992 (after the leaves and seeds had been developing for ~2 weeks); samples were collected every ~20 days throughout the growing season, until the vegetation died in October 1992. Tree bark was collected in July 1991 and February 1992, and soil samples were collected in May 1993.

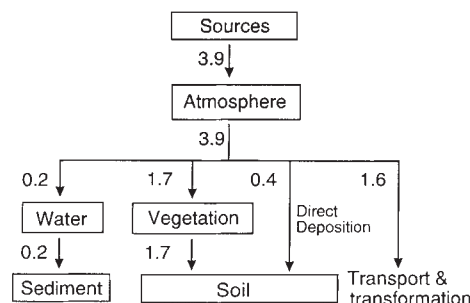
Table 1 shows the sample type, number of samples, average total PAH concentration (sum of all individual PAHs listed above) for the entire growing season, and per cent relative standard deviation for our measurements. The leaf, needle and seed concentrations on an areal basis were calculated based on a measured density of 0.9 g (net weight) vegetation per cm³, an average measured water content of 50%, an average measured pine needle thickness of 0.5 mm, and an average measured leaf and seed thickness of 0.2 mm. The bark concentration on an areal basis was measured directly from the known area of bark removed from the tree.

Table 1 shows that there is a significant difference in the mean concentration between the different types of vegetation, and ~40% relative standard deviation within the vegetation types. The significant differences in the mean concentration can be explained by the differences in plant lipid content. As we have previously reported⁶, vegetation with a high lipid content (such as tree bark and needles) generally has a high PAH concentration, and most of the variation in PAH concentration among the different tissue types can be attributed to varying amounts of lipids. We have also reported⁶ that the equilibrium between the atmosphere and vegetation is temperature dependent, with high vegetation PAH concentrations at low ambient temperatures (spring and autumn), and low concentrations at high ambient temperatures (summer). This seasonal effect results in the observed relative standard deviations of 40% within the different vegetation types.

TABLE 1 Results of PAH measurements at Bloomington, Indiana

Sample	<i>n</i>	AVE _w (ng-PAH per g-vegetation dry wt)	AVE _a (ng-PAH per cm ² -vegetation)	r.s.d. (%)
Leaves	46	567	5.10	42.8
Seeds	12	189	1.70	44.3
Needles	44	893	20.1	43.7
Bark	31	877	229	40.7

Sample is the type of matrix, *n* is the number of samples analysed, AVE_w is the average total PAH concentration on a vegetation dry weight basis, AVE_a is the average total PAH concentration on a vegetation areal basis and r.s.d. is the relative standard deviation of the measurements. METHODS. Leaf, needle and seed samples were cut from the tree, and the bark was collected by chiselling a known area to the depth of ~1 cm. All samples were spiked with five perdeuterated PAH internal standards just before extraction. The leaf, needle and seed samples were extracted in their original state by sonication in dichloromethane; bark samples were ground and extracted with supercritical chlorodifluoromethane. All extracts were cleaned using 2 g silica, solid-phase extraction columns. Recovery of PAHs from all samples was 88–96%, and blank experiments (which were free of PAH contamination) were done with every set of extractions. All samples were analysed by gas chromatographic mass spectrometry using electron impact ionization and selected ion monitoring. The detection limit was ~0.13 ng per g-vegetation dry wt for individual PAHs. Experimental details are given in ref. 6.

FIG. 1 Total PAH flow rates (in units of 10^6 kg yr⁻¹) for the northeastern United States.

In 1983, total PAH emissions from all sources (autos, residential heating, burning, industrial processes and so on) in the United States were $\sim 7.5 \times 10^6$ kg yr⁻¹ (ref. 9). Taking 52% of this number (the percentage of the population of the United States that lives in this region), we calculated PAH emissions into the atmosphere over the region of interest (the airshed) to be 3.9×10^6 kg yr⁻¹. Assuming steady-state conditions, the flow of PAHs out of this airshed is also 3.9×10^6 kg yr⁻¹.

The flow to vegetation from this airshed was calculated by assuming an average vegetation surface area of 7 m² vegetation per m² land; this is the so-called "leaf area index" (refs 10–12), and does not include the surface area contributed by tree bark, plant stems or the understory. We also assumed that 90% of the area in this region has vegetation (in the form of crops, forests, or urban vegetation), and that the average total PAH areal concentrations for vegetation in this region is 8 ng cm⁻² (based on our measurements for leaves and needles shown in Table 1). We calculated this vegetation areal concentration assuming that 78% of the land area in this region is covered by crops, grasslands and urban vegetation that would have a similar lipid content and PAH concentration as the leaf concentrations we measured (5.10 ng cm⁻²). We also assumed that 22% of the land area is forested, and would have a similar lipid content and PAH concentration as the needle concentrations we measured (20.1 ng cm⁻²). Using these values, we calculate the resulting flow of PAHs from the atmosphere to vegetation in this region to be 1.7×10^6 kg yr⁻¹ or 44% of the total PAH flow from the airshed. We estimate the relative standard deviation of this calculation to be $\pm 40\%$ based on those given in Table 1 for our measurements. We realize that this is an estimate based on measurements from a suburban site, but we believe that it is conservative because tree bark, plant stems and the forest understory are not included in the vegetation concentrations or surface area estimation. Clearly, they contain a substantial amount of PAH (Table 1) and provide a substantial amount of surface area.

We assume that the PAHs absorbed by vegetation are incorporated in the soil once a year, when the vegetation falls to the ground at the end of the growing season and decays^{7,8}. Hence the total PAH flow from vegetation to the soil of this region is 1.7×10^6 kg yr⁻¹. Once the PAHs are incorporated in soil they may be biologically degraded, photo-degraded, stored permanently, or a small amount may volatilize back into the atmosphere. Volatilization would be limited to the most volatile, lower-molecular-weight PAHs such as phenanthrene and anthracene. We believe that most PAHs that are absorbed by vegetation when it falls to the ground are permanently removed from the atmosphere.

The total PAH flow to soil (vegetation input plus non-vegetation input) was calculated from the average total PAH flux to soil and the land area, which is 90% of the total area of this region. We calculated the soil flux from four measurements that averaged 2,150 ng cm⁻² soil and an estimated soil storage capacity of 30 years (ref. 7). This is a flux estimate of 72 ng cm⁻² yr,

and it is consistent with literature values^{13–15}. This results in a total PAH flow to soil of $2.1 \times 10^6 \text{ kg yr}^{-1}$. Subtraction of the PAH input from vegetation gives a flow of $0.4 \times 10^6 \text{ kg yr}^{-1}$: in other words, ~10% of all PAHs entering this airshed are deposited directly to soils without partitioning to vegetation. This is reasonable considering that most of vegetation in this region is deciduous, and that the soil is directly exposed to the atmosphere for several months of the year.

The total PAH flow to the Great Lakes water and sediment was calculated by assuming that the average atmospheric PAH flux to water in this region is $50 \text{ ng cm}^{-2} \text{ yr}$ (refs 16–19) and that the area is 10% of the total area of this region. The resulting flow of PAHs is $0.2 \times 10^6 \text{ kg yr}^{-1}$, or ~5% of all PAHs that enter this airshed. Because the PAHs in the water are at steady state, we have assumed that the PAH flux to sediment is the same as that to water. By difference we calculate that $1.6 \times 10^6 \text{ kg yr}^{-1}$ or ~41% of the PAHs that enter the atmosphere do not reach sediments or soils. This may be due to atmospheric transport out of the region, to photochemical transformation^{20,21}, or to reactions with atmospheric O_3 (refs 22–24), NO_x (refs 24–26), or hydroxy radicals (ref. 24). Figure 1 summarizes our calculated flow rates.

Our mass-balance model estimates the average annual removal of PAHs from the atmosphere by vegetation in an area of the world that contains 10% water and 90% land and vegetation. Our model does not detail the temperature dependence we observed between the atmosphere and vegetation, but our previous results⁶ indicate that vegetation is a better sink for PAHs in the spring and autumn (lower ambient temperatures) than it is in the summer (higher ambient temperatures)⁶. Also, the magnitude of the sink due to vegetation will change depending on the amount of vegetation (percentage of total area) available. It is difficult to predict this magnitude on a global scale because little is known about atmospheric PAH deposition to the oceans.

Although we measured the individual PAHs in our samples, we chose to develop this preliminary model on a total PAH basis (sum of all individual PAHs) because it is difficult to predict the source strength of the individual PAHs. But because of their higher lipophilicity, we believe that vegetation plays a bigger role in removing the higher-molecular-weight PAHs (such as benz[a]anthracene to benzo[ghi]perylene) than the lower-molecular-weight PAHs (such as phenanthrene and anthracene) from the atmosphere.

We have used these calculations to estimate the magnitude of vegetation's role in the removal of lipophilic PAHs from the atmosphere, and have based the calculations both on data we have obtained from numerous vegetation and soil samples and on reliable data from the literature; we have also made reasonable assumptions. Based on the variability of the averages we have used and on the uncertainties of our assumptions, we estimate that the precision of the flows shown in Fig. 1 is $\pm 40\%$ (relative standard deviation). Even with this error, our results indicate that vegetation is a major pathway through which lipophilic organic compounds are removed from the atmosphere. These results have significant implications regarding the partitioning of these compounds in the environment and the potential of vegetation to clean the atmosphere. □

Received 4 November 1993; accepted 18 May 1994.

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ACKNOWLEDGEMENTS. This work was supported by the National Institute for Global Environmental Change and the US Department of Energy.

Evidence from the Oman ophiolite for active mantle upwelling beneath a fast-spreading ridge

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It is commonly accepted that as oceanic lithosphere cools, it slides away from the spreading centre under its own weight¹. It has been suggested^{2–5} that even at the spreading axis this process controls the dynamics, causing asthenospheric mantle material to rise passively before being dragged away from the ridge by the overlying lithosphere. Alternatively, the upwelling of the asthenosphere at the ridge might be 'active', caused by the buoyancy of partial melt^{6–10} or of the less dense peridotite left behind by such melting^{11,12}. At slow-spreading ridges, 'bull's-eye' gravity anomalies^{13,14} have been interpreted as suggesting focused accretion, and hence active mantle diapirs. At fast-spreading ridges, however, these anomalies are weaker and less clearly three-dimensional¹⁵, which may reflect passive mantle upwelling¹⁶. Here we show that structural data from the Oman ophiolite, which is thought to have been created at a fast-spreading ridge^{17,18}, are consistent with active mantle upwelling beneath the ridge, and not with models of passive flow. The absence of well marked bull's-eye anomalies at fast spreading ridges may be explained by their relatively constant crustal thickness and near-isostatic equilibrium.

The Oman ophiolite represents the largest and least altered piece of oceanic lithosphere obducted onto a continental margin. Several studies have concluded that it was derived from a fast-spreading oceanic ridge^{17,18}. Uranium-lead dating has revealed age differences of the order of only 2 million years over across-strike distances exceeding 100 km across the Oman ophiolite, suggesting a fast-spreading rate¹⁹. The 2–4 km thickness of the layered gabbro sequence and its continuity throughout massifs 50 km in dimension or more imply the existence of permanent magma chambers, a feature met so far only in fast-spreading ridges. The study of the segmentation along the palaeo-ridge strike of this ophiolite¹⁷ has led to the discovery of only one transform zone which, by its width (20 km) and high-temperature character, fits better with a fast-spreading environment than with a slow one²⁰. Furthermore, along-strike variations in the sheeted dyke complex reveal an overall style of segmentation

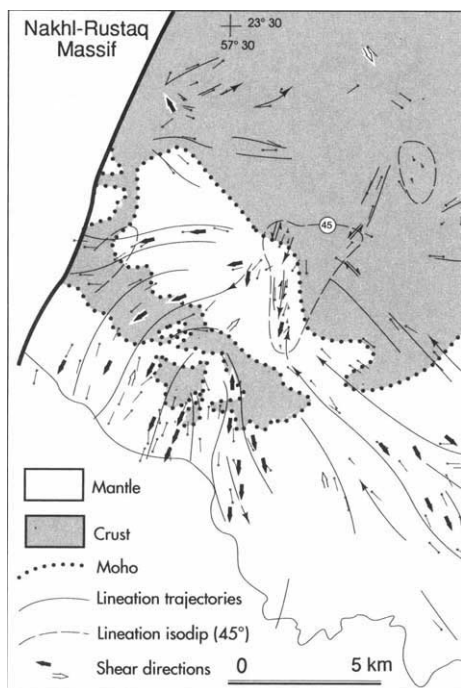


FIG. 1 Mineral lineation trajectories showing the parallelism between plastic flow lines in the uppermost mantle and magmatic flow lines in the crustal layered gabbros around a diapiric area partly concealed below layered gabbros. Flow lines diverge away from the presumed diapiric centre; shear direction analysis reveals a dominant top-away-from-the-diapir shear. Short black lines represent individual lineation measurements.

very similar to that of fast-spreading ridges such as the East Pacific Rise, with second-order discontinuities that probably represent overlapping spreading centres spaced on average 50 km apart along the strike of the Oman axis¹⁸. Finally, in a comprehensive study of ophiolites¹⁷ taking into account a large number of structural and petrological data, Oman ranks as an extreme case, which by comparison with similar oceanic data, evokes an especially fast-spreading environment.

In the mantle section, deformation occurring at or above solidus conditions, which must therefore be ascribed to asthenospheric mantle flow below an oceanic spreading centre, can be discriminated from later, lower-temperature deformation^{21,22}. Such high-temperature deformation typically produces mineral foliations and lineations in the peridotite, which are defined by the preferred flattening and alignment of olivines, pyroxenes and spinels. This structural information can be converted into kinematic information²³, from which it is possible, from maps of regularly spaced individual measurements, to construct maps of trajectories of asthenospheric mantle flow below a palaeo-spreading centre. Areas of steep foliation thus identified, associated with steeply plunging stretching lineations, reflect localizing vertical flow and have been related to mantle diapirs; in contrast, flat-lying foliations, with lineations fanning out from the presumed diapirs, have been related to mantle flow diverging away from the ridge axis^{21,24}. Such mantle diapirs, mapped in several ophiolites²⁵ and best exposed in Oman, are comparable to the Atlantic bull's-eyes. But progress in structural mapping in the southeastern part of the Oman belt suggests that, at the Moho level, diapirs may be altogether smaller (10 km across), better aligned along the presumed ridge axis and more numerous (three over a 70 km distance) than previously thought; this may indicate that diapirs were detached from some linear asthenosphere upwelling at shallower depth than first suggested^{6,7,17}. As in the mantle, trajectories of mineral foliations and lineations can be mapped in the layered gabbros of the crustal section; in Oman,

they can be ascribed to magmatic suspension flow (that is, a mush in which grains move around each other) rather than to solid-state flow because of the lack of intracrystalline plastic strain in the minerals^{26,27}. From these studies, we restate here the following points.

(1) Flow structures, especially lineations, are in most cases remarkably parallel below and above the Moho. In the lower layered gabbros, the magmatic foliation and layering are parallel both to the Moho surface and to the plastic foliation in the dunites and harzburgites from below the Moho. The magmatic mineral lineations in these gabbros are similarly parallel to the plastic mineral lineation in harzburgites and dunites. This striking result has been confirmed everywhere in the Oman ophiolite as shown by general and local structural maps^{22,24} (Fig. 1). The angles between magmatic lineations in gabbros, and plastic lineations of the immediately underlying mantle rocks plotted in Fig. 2a are small, probably within the error range of measurements. In the trajectory map²² as well as in Fig. 2b, it is also seen that both sets of lineations strike at any angle with respect to the average northwest-southwest or north-northwest-south-southwest ridge direction deduced from the azimuth of the sheeted dike complex. In particular, around diapiric areas these lineations tend to diverge in all directions (Fig. 1).

(2) Horizontal mantle flow just below the Moho reflects a very large plastic strain, much larger than at greater depth. In this dominantly shear flow, an inversion of the shear direction is commonly recorded at a few hundred metres below the Moho. This shear inversion is present in several other ophiolites¹⁷ and has been observed and mapped in several places along the mantle section of the Oman ophiolite^{21,28,29}.

(3) Layered gabbros in the lower crust have crystallized during magmatic flow. The flow took place in a progressively cooler and more concentrated crystal mush, and has resulted in the development of structures very similar to typical solid-state structures in metamorphic rocks²⁷. In such 'magmatic tectonites', some structures such as sheath folds or boudins indicate that the flow was large and others, such as magmatic shear

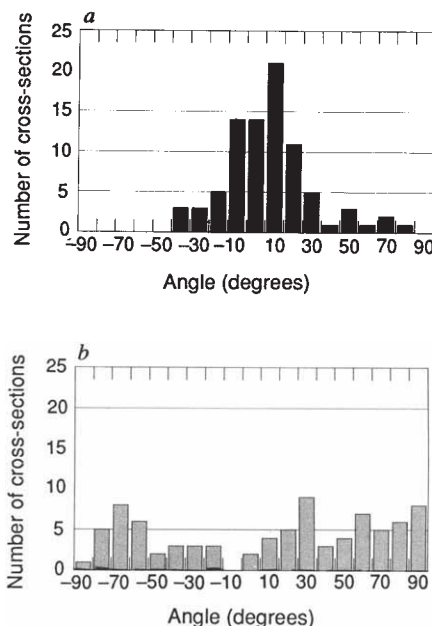


FIG. 2 a, Histogram showing the general parallelism between mantle lineations and gabbro lineations across the Moho. The histogram shows averaged lineation measurements from 83 cross-sections throughout the Oman belt. Lineations at some distance from the Moho contribute to the dispersion, as does faulting localized on the Moho surface in some of the cross-sections. b, Histogram showing, at the same measurement locations as in a, the absence of a relation between the mantle lineations and the trend of the sheeted dikes.

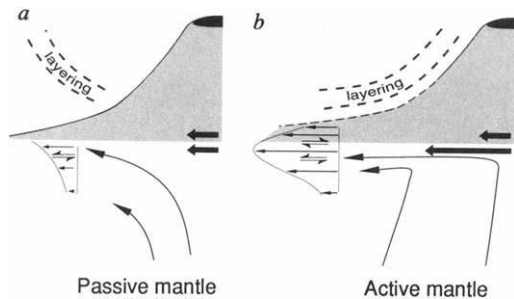


FIG. 3 Schematic half cross-sections of the predicted upward flow (thin black arrows) and the velocity profiles in the uppermost mantle and the deep crust at an oceanic ridge for passive (two-dimensional) mantle uprise (a) and active (three-dimensional) mantle uprise (b). Light grey indicates magmatic mush and black indicates the melt lens. The dip of the foliation in the layered gabbros is indicated for both situations (see text for further discussion). In the active case (b), note the shear inversion related to the forceful flow at the edge of the diapir; the plane of shear inversion is also a plane of no deformation. The large black arrows show the relative global velocity of the crust and the upper mantle in both situations.

bands on various scales, indicate that it was still operative during solidification of the mush, being progressively localized into more widely spaced shear bands.

Assuming passive mantle flow beneath ridges, the magmatic deformation in the gabbros has been explained by subsidence of the crystalline mush deposited on the floor of a perched magma lens^{4,5}. In this model, the flow and strain fields are such that the gabbro foliations are inclined toward the magma chamber axis (Fig. 3a), and the lineations in both the crustal gabbros and the mantle peridotites are expected to be parallel to the spreading direction because passive mantle upwelling and spreading is necessarily two-dimensional¹⁶. The passive spreading model can explain a general parallelism between mantle and crustal lineations, but fails to explain their common and close orientation at any angle to the ridge direction and divergence away from areas of steeply plunging lineation (Figs 1 and 2b). From this it follows that the active mantle structure is not two- but three-dimensional, and that it takes the form of diapirs spaced on a scale of a few tens of kilometres.

Furthermore, the passive model cannot explain the inversion of the shear direction recorded in several areas of Oman^{21,28,29}. In contrast, active spreading accounts for the shear inversion in the mantle section (Fig. 3b). Inversion is the result of the forceful horizontal flow of asthenospheric material, several times faster than the spreading rate, induced by the underlying buoyant uprise^{6,7,30}.

Finally, the requirement in the passive flow models^{4,5} for gabbro foliations at and near the Moho to be inclined toward the ridge axis is not verified by field investigations in Oman: in the lowermost gabbros, foliation and layering are closely parallel to the Moho and, up-section, their inclinations dip away from the diapiric centres rather than towards them (Fig. 3b)^{17,24}.

The active and three-dimensional mantle uprise below fast-spreading ridges, as illustrated in the Oman ophiolite, must of course be reconciled with the weak circular gravity anomalies detected at such ridges¹⁵, which are more in favour of a two-dimensional mantle upwelling. The apparent contradiction may be explained in two ways. Recent work¹⁴ indicates that the shape and magnitude of the Bouguer anomaly on the Mid-Atlantic Ridge may be explained entirely in terms of crustal thickness variations; the absence of such anomalies along the East Pacific Rise would then reflect only the relatively constant crustal thickness in the fast-spreading ridge environment rather than the absence of focused mantle upwelling. Furthermore, fast-spreading ridges are near isostatic compensation because of their high temperature; a weaker gravity signal is thus expected. In fact, a recent gravity survey along a segment of the East Pacific Rise

shows that the signal does vary along strike, with minimum values near the centre of the segment, consistent with focused three-dimensional mantle upwelling³¹. □

Received 8 December 1993; accepted 19 May 1994.

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ACKNOWLEDGEMENTS. We thank N. Sleep and C. MacLeod for reviews of this manuscript. Field work in Oman has been possible thanks to the local support from the Ministry of Mines and Petroleum and to financial support from the CNRS.

Evolution of phenotypic optima and copula duration in dungflies

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THERE is growing evidence that adaptation may relate to precise conditions of an individual's phenotype, though previous studies have concentrated on discontinuous variation¹. We show here that the copula duration of male dungflies, a widely cited example of optimality in biology^{2–5}, is tuned precisely and continuously to the size of the copulating male. During copulation, new sperm displace previously stored sperm at a rate that shows exponentially diminishing returns⁶. Thus at the optimal copula duration a male's paternity gains from continued copulation drop below that expected from searching for a new female. Small males have lower rates of sperm displacement⁷; they also have less prospect of copulation by take-over of females from other males^{8,9}. Both effects predict that small males will copulate for longer, although displacement differences exert much the stronger influence. Observed copula durations are negatively correlated with size and concur with the optimality model across the natural male size range. This represents one of the first quantitative demonstrations of optimal responses covarying with a continuous aspect of phenotype. Phenotype-limited optimality profiles pose interesting evolutionary questions.

The reproductive behaviour of dungflies, *Scatophaga stercoraria*, has been widely analysed using ideal free^{10,11} and marginal

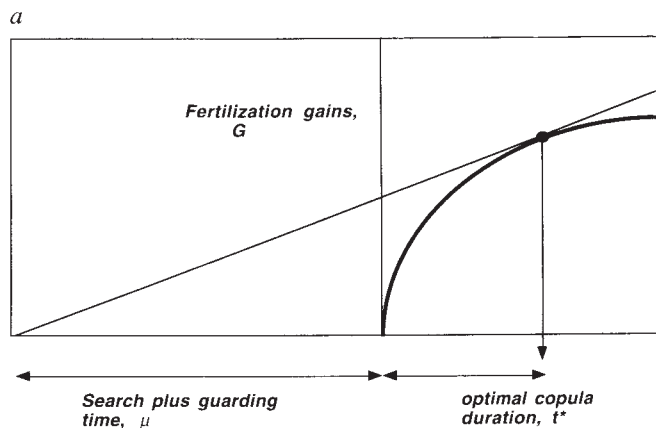


FIG. 1 a, Graphical solution for optimal copula duration in dungflies (modified from ref. 12). The optimal copula duration, t^* is found by constructing a tangent as shown to the curve of expected fertilization gains, G , with copulation time t . Formally, this solution is where

$$\frac{dG(t^*)}{dt} = \frac{G(t^*)}{\mu + t^*} \quad (1)$$

Note that increasing μ increases t^* . The mechanism of sperm competition in dungflies fits a model where seminal fluid flows out from the male at a constant rate and displaces an equal volume of fluid from the female's sperm stores; there is immediate mixing of sperm store contents⁶. It generates an expected gain curve which shows exponentially decreasing fertilization returns^{6,12}:

$$G(t) = G_{\max}[1 - \exp(-ct)] \quad (2)$$

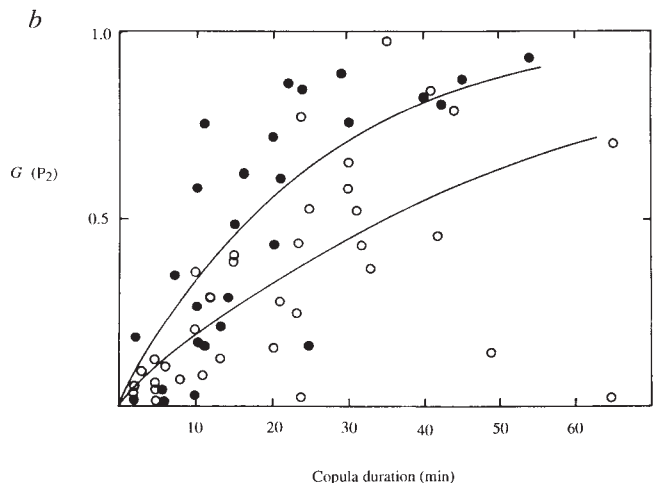
where c = the proportionate rate of sperm displacement, defining the rate at which $G(t)$ rises to its asymptote, $G_{\max}$. Substituting (2) into (1) gives¹⁴

$$\exp(-ct^*) = c(\mu + t^*) + 1 \quad (3a)$$

from which t^* can readily be iterated numerically, though the following explicit form gives an excellent approximation²⁰:

$$t^* \approx \left(\frac{1}{c}\right) \left(1 + \frac{1}{c\mu + 1}\right) \ln(c\mu + 1) \quad (3b)$$

Hence $G_{\max}$ does not effect the optimal copula duration, which depends only on μ and c . Note that this solution ignores the effect of take-overs, and assumes that all matings are with incoming females. b, Gain curves, $G(t)$, for large and small males. We collected males from the field and



divided them into large, medium and small size classes. Only the large and small size classes were used in our experiments (the mean $\pm$ s.e. cube of hind tibia length (HTL)³ for large males was 33.46 ± 1.09 versus 13.6 ± 0.42 for small males; $t = 18.35$, d.f. 88, $P < 0.001$). All copulations were done by placing a single female into a 100 ml jar with a smear of fresh dung on a piece of filter paper. A male was then introduced into the jar and the resulting copulation timed. The ambient temperature during copulation was noted and durations were corrected to a standard temperature of 20°C ^{7,21,22}. Initially, virgin females were allowed to copulate with either a large or a small male. First males were allowed to copulate to completion (> 32 min), thereby ensuring that the females sperm stores were filled^{6,22}. The gain (G) for large or small second males was then estimated by interrupting their copulations after periods of 2 to 60 min. G was calculated as the proportion of eggs fertilized by the second male to mate (the P_2 value⁶), using the standard irradiated-male technique^{7,15}. The data were fitted to the model of equation (2) with $G_{\max} = 1.0$, by least-square deviation. The data for both large (closed symbols) and small (open symbols) males gave significant fits to the model (large males: $1 - \exp(-0.041t)$; $F_{(1,26)} = 210.66$, $P < 0.001$, $r^2 = 0.89$; small males: $1 - \exp(-0.020t)$; $F_{(1,34)} = 95.84$, $P < 0.001$, $r^2 = 0.74$). Furthermore, these curves differ; the displacement rate, c , was greater for large males than for small males (0.041 ± 0.005 versus 0.020 ± 0.003 ; $t = 10.22$, d.f. 60, $P < 0.001$). These different values for c yield predicted optimal copula durations from equation (3) of 55 min and 89 min for the large and small males, respectively (ignoring the effect of take-overs). Note that the mean size of 'large' and 'small' males in this experiment were both smaller than the average male size (see Fig. 3a).

value models^{12,13} in behavioural ecology. Dungfly males arrive quickly at fresh cattle droppings and copulate when they capture incoming gravid females. Females contain sperm from previous matings¹⁴ and as copulation proceeds, new sperm displace previously stored sperm at a decreasing rate^{6,12}. After copulation, the male guards the female while she oviposits in the dropping: he thereby ensures that his sperm are not displaced.

The male's fertilization gains, G , depend on how long (time t) he has copulated, that is, on his sperm representation in the female's store. The optimal time to copulate, t^* , is determined by the marginal value theorem^{12,13}: a male should leave when his marginal gains from mating drop to the expected gain from the habitat as a whole. Calling μ the mean time taken to find and guard an incoming female (which appears constant, regardless of population density¹⁰), t^* is shown graphically in Fig. 1a.

Earlier analyses of optimal copula duration in dungflies^{12,14,15} aggregated all male size classes, and showed that the mean observed copula duration of 36.5 min was quantitatively close to that predicted by equation (3) of Fig. 1a legend; 42.5 min¹⁴. The mean displacement rate, c , is 0.061 (ref. 6); roughly 1/16 of stored sperm are displaced per minute of copulation. From field estimates, $\mu = 156.5 \text{ min}^{10}$.

An experiment using males of two size classes clearly demon-

strated that large males experience different gain curves from smaller males (Fig. 1b) because they have a higher rate of sperm displacement. This will affect optimal copula duration: if all males experience the same mean time, μ , to find and guard a female, then smaller males should copulate longer than larger males because the tangent line will touch the gain curve at a higher value for t^* . Because c changes across the natural range of male sizes (Fig. 2) we would expect copula duration to be size dependent.

However, we can identify a second reason why copula duration should be size dependent. In about one third of ovipositions, 'take-overs' occur where an attacker displaces the paired male and copulates, gaining most of the remaining eggs¹⁵. Take-overs are size dependent: a larger male takes a female from a smaller male, with a probability increasing with size disparity⁹. Take-overs have two principal effects: (1) they decrease the asymptote, $G_{\max}$, of the gain curve for both winning and losing males, by reducing the expected number of eggs gained, and (2) for winning males they significantly reduce μ , the time to find and guard a female. Effect (1) will not influence optimal copula duration for either males when $G_{\max}$ is the mean across all matings (winning males appear not to discriminate between 'take-over' and 'incoming-female' matings¹⁶), because $G_{\max}$ does not affect t^*

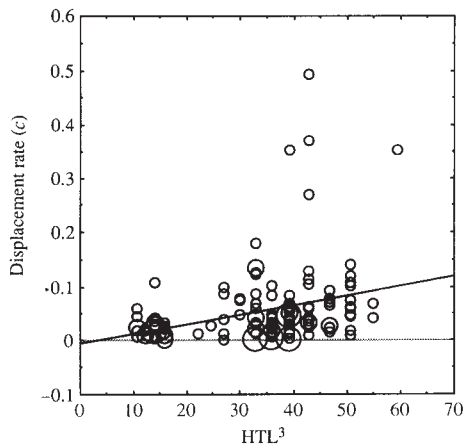
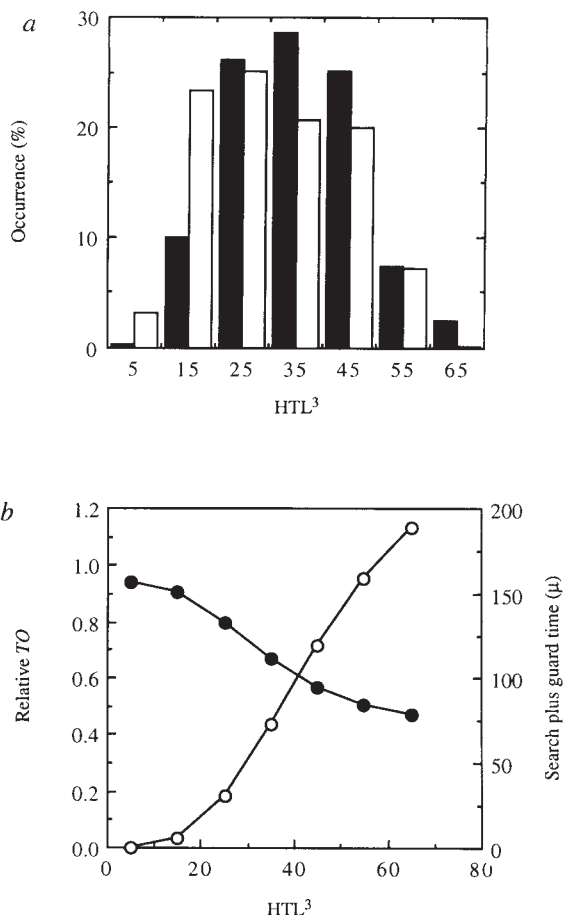


FIG. 2 Relationship between sperm displacement rate and male size. We estimated gains, G (proportion of eggs fertilized by the second male to mate) as in Fig. 1 but for a group of males that showed both continuous variation in size (HTL^3) and which were all allowed to copulate until completion. G values were used to estimate each male's displacement rate, c , using the following rearrangement of equation (2) with $G_{max} = 1.0$: $c = [-\ln(1 - G)]/t$. The regression of c against male size (HTL^3) was significant, $F_{(1,135)} = 14.27$, $P < 0.001$, $r^2 = 0.10$; slope ($\times 10^{-2}$) = 0.18 ± 0.05 , intercept ($\times 10^{-2}$) = -0.3 .

FIG. 3 Estimation of the effect of take-overs on the mean time taken to find and guard a female, μ , experienced by males of different sizes. Consider first the effect of winning take-overs: this decreases (1) the interval between successive matings, and (2) the mean time spent guarding (females mated after take-over have fewer eggs). a, Frequency distributions of paired (solid bars, $n = 370$) and searching (empty bars, $n = 418$) males of different sizes at the droppings, summated for the year 1978 from the data of ref. 16. Let s_i = the frequency of searching males of size i , and p_j = the frequency of paired males of size j . These distributions were used to calculate the probability, P_{ij} , that a searching male of size i attacks a paired male of size j : $P_{ij} = s_i p_j$. Should a struggle result between i and j , the probability of a take-over of j by i , V_{ij} , is estimated from the result of ref. 9 that $V_{ij} = 0.64 - 0.55 (HTL_i^3 / HTL_j^3)$, subject to the finding that no take-over occurs if i is smaller than j . A measure of the relative gains of take-overs summed for all males within each of the searching male size classes was found by summing: $Q_i = P_{ij} V_{ij} + P_{ik} V_{ik} + \dots$ and so on. However, Q_i has no absolute magnitude; we need an estimate of the frequency of take-overs in the field. We therefore converted Q_i into T_i : the expected number of take-overs per mating with an incoming female, summed for all males in size class i . In the field, take-overs are almost entirely restricted to the oviposition phase. The probability that a given female experiences a take-over during her visit to the dropping is 0.36^{15} . Hence $T_i = Q_i (0.36 / \Sigma Q_i)$. But the number (probability density) of males in size class $i = s_i$; to obtain the number of take-over matings won per male of size class i we must divide T_i by s_i . So, in *b* we plot $TO_i = T_i / s_i$ (unfilled symbols). The smallest males win virtually no take-overs, but largest males will gain about one take-over mating for every mating with an incoming female. They therefore experience a reduced time to find and guard a female, μ . On average, a male expects to find and guard an incoming female every 156.5 min^{11} . To include take-overs, the average time to find and guard a female (filled symbols) for the various male size classes were calculated as $\mu_i = (140 + 16.5(1 + 0.570 TO_i)) / (1 + TO_i)$; 140 min is the search time to find a female (which halves if there is one take-over for every mating with an incoming female) and 16.5 min is the guarding time (females that are taken-over will on average be half way through oviposition and will require only half the guarding)¹¹. Thus a large male obtaining one take-over for each mating with an incoming female has an average μ of 82.4 min ($(140 + (16.5 + 8.25))/2$), roughly half that of a small male which gains matings only with incoming females ($(140 + 16.5)/1$). The method outlined involves several simplifying assumptions, but should give a good approximation of μ_i . A major assumption (for which there is good evidence²²) is that males treat incoming and take-over females equally; if males could discriminate, shorter copulations would be predicted after take-overs. Now consider the effect of losing in take-overs:

(equation (3), Fig. 1). But effect (2) is important: the reduction in μ is greater for large males because they gain many more take-overs. The quantitative analysis of this effect is complex (Fig. 3), involving knowledge of the distribution of male size (Fig. 3a) to calculate relative numbers of take-overs for each male size (Fig. 3b). Biggest males experience a μ of roughly half that of smallest males, for which μ is around 156.5 min , because they gain virtually no take-overs (Fig. 3b). Again, smaller males should therefore copulate longer than large males.

We examined copula duration in relation to male size and found that, as predicted by both sperm displacement and take-over variations, copula duration does indeed decrease with male size (Fig. 4A). The copula duration predicted by each variable separately is shown in Fig. 4B: displacement rate clearly exerts a much stronger influence than take-overs. The two effects together (Fig. 4C) give a very good fit to the observed profile across most of the natural size range, and the take-over effect reduces the predicted copula duration for averaged-sized males almost exactly to the observed mean copula duration. Predicted copula durations for very small males tend to be higher than those observed. Although the model fit appears to deteriorate at the lowest end of the size distribution, this region is at the extreme and beyond the normal size range. Further, extremely long copulations may be penalized by constraints not included in the model (such as risks of predation and not completing copulation before dusk; deterioration of the dung surface for oviposition). Our analysis assumes that all males have equal



this is much simpler. Losing does not affect the interval between successive matings, but reduces the time spent guarding (guarding terminates on take-over). For simplicity, because this affect can generate only trivial variation in μ across the male size range, it is omitted.

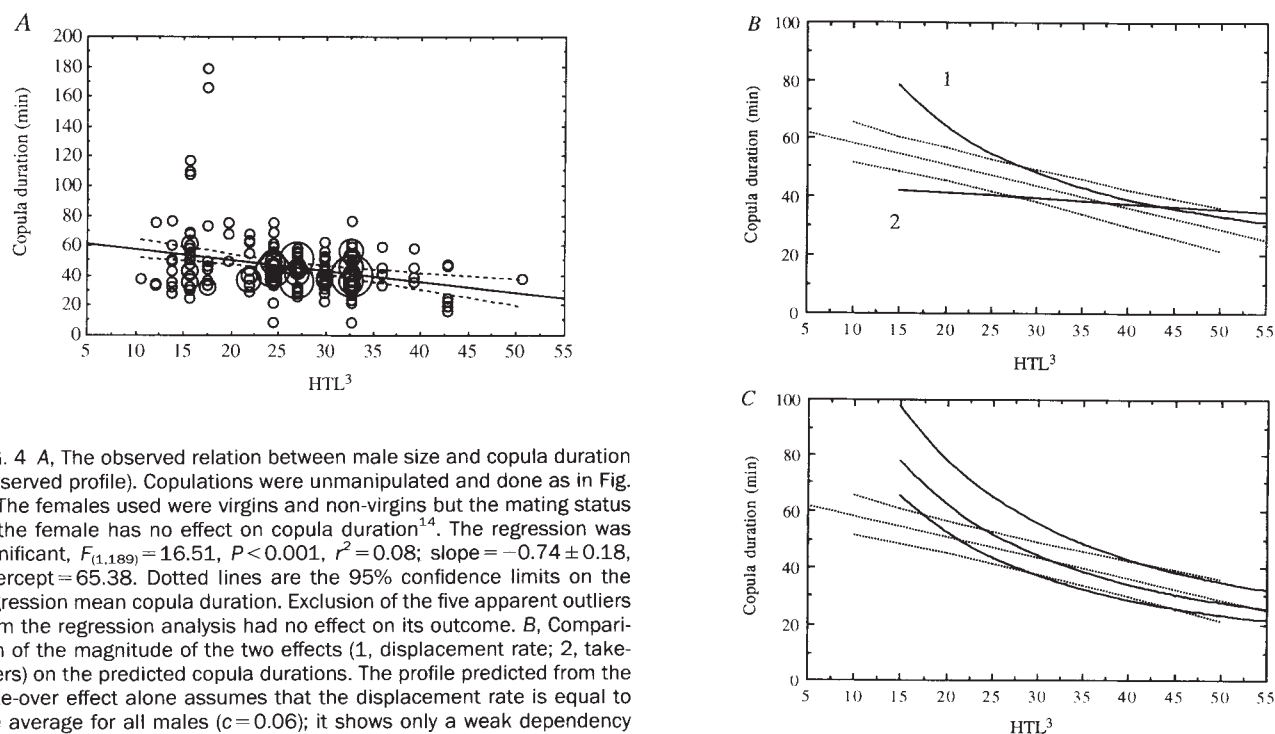


FIG. 4 A, The observed relation between male size and copula duration (observed profile). Copulations were unmanipulated and done as in Fig. 1. The females used were virgins and non-virgins but the mating status of the female has no effect on copula duration¹⁴. The regression was significant, $F_{(1,189)} = 16.51$, $P < 0.001$, $r^2 = 0.08$; slope = -0.74 ± 0.18 , intercept = 65.38. Dotted lines are the 95% confidence limits on the regression mean copula duration. Exclusion of the five apparent outliers from the regression analysis had no effect on its outcome. B, Comparison of the magnitude of the two effects (1, displacement rate; 2, take-overs) on the predicted copula durations. The profile predicted from the take-over effect alone assumes that the displacement rate is equal to the average for all males ($c = 0.06$); it shows only a weak dependency on male size. The profile predicted from the displacement effect alone assumes that searching plus guarding times (μ) are equal to the average for all males for matings with incoming females (156.5 min), as in earlier analyses. It shows a strong dependency on male size, particularly for males below average size. Predicted profiles were calculated using equation (3b). The observed profile and 95% confidence limits are shown. C, Predicted optimal profile; both effects included, calculated

using equation (3b). The error limits on the predicted profile are conservative, because they were derived using only the $\pm$ s.e. of the slope of c (Fig. 2). However, the effects of take-over are so weak that the contribution of error due to the calculation of the take-over effect would be very small. The observed profile and 95% confidence limits are shown for statistical comparison.

capture rates of incoming females: smaller males tend to search in the surrounding grass^{8,16} but gain rates are roughly equal in all search areas¹⁷. Differences in μ would anyhow exert only a very weak effect (Fig. 4B).

A final reason why size could affect copula duration relates to costs of replenishing the sperm reservoir, due to time spent feeding away from droppings¹⁸. Optimal time feeding may depend on male size, this in turn can affect optimal copula duration. Experiments showed that larger males have bigger reservoirs, but the proportionate rate of filling is independent of size (unpublished data). Calculation of the effect this has using an extended model for copula duration¹⁸ shows any size dependency to be insignificant. In summary, the principal effect of size on copula duration derives from size differences in the rate of sperm displacement, though take-overs also exert some effect, and feeding differences virtually no effect.

The present study represents one of the first quantitative demonstrations that an observed phenotypic profile approxi-

mates to optimality predictions. The evolution of adaptive profiles requiring behaviour to be continuously conditional on phenotype poses certain evolutionary problems. The underlying genetic mechanism may range in the continuum between (1) having n genes, one for each phenotype, of which only the relevant gene is expressed in a given individual while the other ($n - 1$) remains inactive; or, more plausibly (2) having a differential response to some general determinant, such as hormonal titre¹⁹, which covaries continuously with phenotype. In (2), selection must tune the correct adaptive responses to each phenotypic state, presumably by the action of modifiers. Any change in the 'determinant profile' is likely, at least initially, to alter (maladaptively) any other 'response profiles' tuned to it. Adaptation is therefore more likely to alter the local reaction to the determinant, that is, modifiers tune the sensitivity of each phenotype, rather than change the determinant profile itself. Continuous adaptive profiles may therefore involve complex genetic mechanisms. □

Received 2 December 1993; accepted 12 May 1994.

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ACKNOWLEDGEMENTS. We thank E. L. Charnov for insights that have vastly improved this paper. This work was supported by a NERC grant and a SERC Senior Fellowship to G.A.P. and a SERC Advanced Fellowship to L.W.S.

A geometric process for spatial reorientation in young children

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DISORIENTED¹⁻³ rats and non-human primates reorient themselves using geometrical features of the environment^{2,4-6}. In rats tested in environments with distinctive geometry, this ability is impervious to non-geometric information (such as colours and odours) marking important locations and used in other spatial tasks⁷. Here we show that adults use both geometric and non-geometric information to reorient themselves, whereas young children, like mature rats, use only geometric information. These findings provide evidence that: (1) humans reorient in accord with the shape of the environment; (2) the young child's reorientation system is impervious to all but geometric information⁸, even when non-geometric information is available and is re-presented by the child—such information should improve performance and is used in similar tasks by the oriented child; and (3) the limits of this process are overcome during human development.

All organisms that navigate must reorient themselves when they lose track of their position and heading. Although young children use non-geometric landmarks to locate objects when their sense of orientation is intact⁹⁻¹², no previous study has examined children's or adults' reorientation by geometric and non-geometric information or has compared these abilities with those of other species. In our experiments, subjects saw an object being hidden in a corner of a rectangular room and then were disoriented. Orientation was partially specified by the room's shape and fully specified in some conditions by non-geometric landmarks. Subjects demonstrated their ability to reorient themselves by locating the hidden object.

In experiment 1, university students were tested in an all-white room and in a room with one blue wall (Fig. 1). Unlike rats, human adults used the non-geometric landmark ($F(1, 14) > 35$,

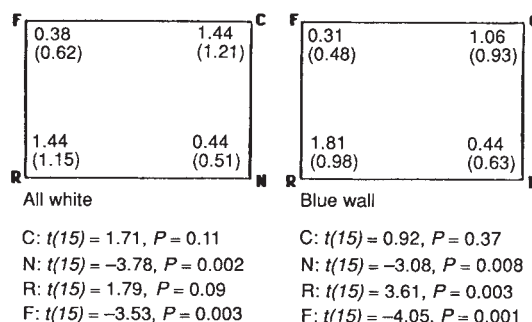


FIG. 2 Search patterns for experiment 2 with young children ($n = 16$, mean age 20.9 months). The experimental design, procedure and analysis were the same as before except that each child's parent (blind to our hypotheses) conducted the experiment from inside the chamber, hiding a toy brought from home. To disorient the children, parents lifted them, covered their eyes, turned at least 4 revolutions, and released them on cue from the experimenter facing a randomly pre-chosen wall. Care was taken to avoid subject dizziness and to ensure that parents did not cue subjects to any location. Only subjects completing 3 or 4 trials were included. Five additional subjects were eliminated for failure to complete 3 trials or for experimenter error; their data were similar to those of the other subjects. Subjects did not search the correct corner in the blue-wall condition more than the correct corner in the all-white condition ($t < 1$). The ANOVA revealed a significant effect of geometry ($F(1, 14) = 31.43, P < 0.001$) but no effect of condition, proximity or sex, and no significant interactions among these factors. No effect of training was found in the blue-wall condition by a Mann-Whitney test comparing subject accuracy in the first and last search trials, $P > 0.20$, despite the fact that children were rewarded for success on their first guess and corrected for failures. This suggests that children's search is not easily modified by feedback. A further experiment (manuscript in preparation) showed that children's failure to use non-geometric information for reorientation does not result from the disorientation procedure itself (Fig. 4 legend).

$P < 0.001$). In the white room, subjects exclusively searched the two geometrically appropriate corners, choosing the correct and rotationally equivalent corners equally often ($t(15) = 1.33$). These results indicate that the disorientation procedure was effective and that adults used the metric and sense properties of

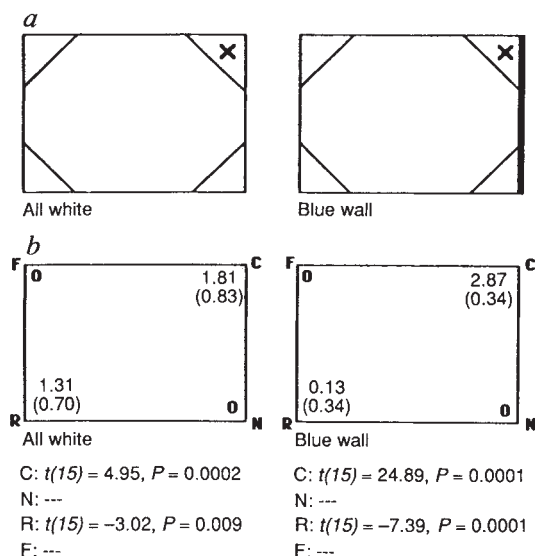


FIG. 1 Testing conditions and search patterns for experiment 1 ($n = 16$). a, The enclosed rectangular 6.25 × 4 × 6.25-foot testing room was white, with lights centred above each wall, a concealed entry, 4-foot-tall red corner panels, and a bright blue 4 × 6.25-foot cloth covering (bold line). Subjects disoriented themselves by covering their eyes, inertially rotating at least 10 revolutions, and stopping on cue from the experimenter facing a randomly pre-chosen wall. A central overhead video camera recorded subjects' searches for subsequent coding by an observer blind to hiding location. A central overhead white-noise generator prevented subjects from orienting by use of any extraneous sound beacon. Hiding location, side of blue wall, order of search sessions, and subject sex were counterbalanced across subjects. Subjects completed 3 or 4 trials per condition with a single hiding location. b, Mean search frequency (and standard deviation) at each corner (C, correct; R, rotationally equivalent; N, near but geometrically inappropriate; F, far but geometrically inappropriate), and summary statistics for search at each position. For the single-sample t-tests, chance is 25% of mean search frequency per subject. All P values are two-tailed. Search frequencies at each corner were analysed by a 2(sex) × 2(condition: all-white or blue-wall) × 2('geometry': search at C and R versus N and F) × 2('proximity': search at C and N versus R and F) mixed-factor ANOVA. There were significant main effects of geometry and proximity, significant interactions of each of these effects with condition, and a significant three-way interaction of condition, geometry and proximity (all values of $F(1, 14) > 35, P < 0.001$), reflecting subjects' use of both geometric and non-geometric information to guide search in the blue-wall condition. Search at C in the blue-wall condition exceeded search at C in the white room ($t(15) = 4.58, P < 0.001$). For all other effects, including those involving sex, $P > 0.10$.

the room to reorient themselves. With the blue wall, search at the correct corner exceeded search at its rotational equivalent ($t(15) = 16.10$, $P < 0.001$). Use of this landmark surpassed mature rats' use of olfactory and visual landmarks in a similar study².

In experiment 2, children aged 18–24 months completed the same task (Fig. 2). In the white room, children searched geometrically appropriate corners ($t(15) = 4.77$, $P < 0.001$) and searched the correct corner and its rotational equivalent equally often ($t(15) < 1$). Unlike adults, children showed the same search patterns with the blue wall (all $F(1, 14)s < 2$). Although they searched geometrically appropriate corners ($t(15) = 3.88$, $P < 0.001$), they did not search the correct corner more than its rotational equivalent and did not search the two appropriately coloured corners more than the other corners (all $ts < 1$). Children's choice of the correct corner in the landmark condition differed from that of adults in experiment 1 (unpaired $t(30) = -7.05$, $P < 0.001$) and was comparable to that of rats tested under similar conditions ($\bar{X}_{\text{rats}} = 0.35$, $\bar{X}_{\text{children}} = 0.31$) (ref. 2).

Experiment 3 investigated whether children could use solid objects as landmarks and benefit from a more elaborate attention-drawing procedure. Subjects participated in two search sessions with non-geometric landmarks (Fig. 3). Before the first session, the experimenter pointed out the landmark(s) to be used in that session. Before the second session, the child and parent played with the landmarks before placing them in the room together with the experimenter. Again, children failed to use the landmarks to reorient themselves.

Further analyses tested whether children failed to use non-geometric information because of a tendency to search corners within their visual field immediately after disorientation. Comparisons of search at either of the two immediately visible corners with search at either of the opposite corners revealed no difference in search rates for either condition (each $t < 1$). After disori-

entation, children often looked about the chamber before searching, but their choice was unaffected by non-geometric information.

Finally, in experiment 4 we investigated whether children's search errors resulted from a perceptual salience hierarchy favouring geometric over non-geometric information, by comparing children's performance on two search tasks (Fig. 4). In the reorientation task, two visually distinct containers appeared in diagonally opposite corners of the room, the object was hidden in one container, and the child was disoriented before search. In the other task, the containers were placed at the room's centre, the object was hidden inside one container, the child closed her eyes but remained oriented, and the containers were moved to the corners before search. Both tasks therefore presented the same object search demands in the same environment, but only the first task required that children reorient themselves. Disoriented subjects searched equally at the two corners, but subjects whose orientation remained intact searched the correct corner more often than its rotational equivalent. Performance in the two conditions differed reliably ($F(1, 28) = 17.51$, $P < 0.001$), suggesting that encapsulation of the child's reorientation process, and not a salience hierarchy favouring geometric cues, accounts for children's search failures.

In summary, young children oriented themselves by analysing the shape of the environment. They failed to orient themselves in

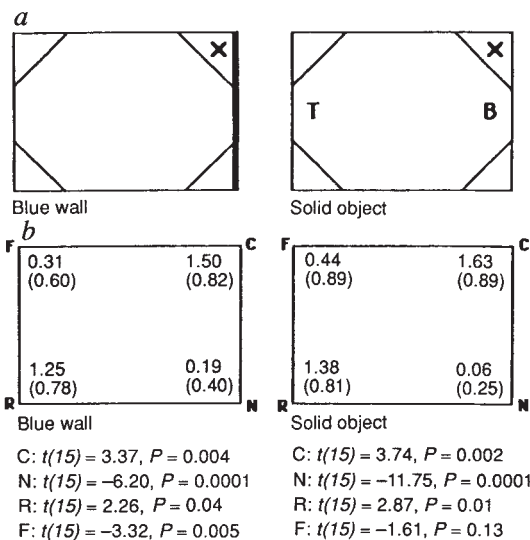


FIG. 3 Testing conditions and search patterns for the children ($n = 16$, mean age 21.2 months) in experiment 3. *a*, The design, procedure and analyses were the same as in experiment 2 except that children were tested once with the blue wall and once with a toy truck (T) and bear (B) with similar global dimensions, placed in symmetrical locations within the room (see text for description of procedures for drawing children's attention to the landmarks). *b*, Mean search frequencies (and standard deviations) for each landmark condition, collapsed across attention-drawing procedures. The analysis revealed a significant effect of geometry ($F(1, 14) = 41.34$, $P < 0.001$), an effect of proximity $\times$ geometry ($F(1, 14) = 6.67$, $P = 0.02$), and no other effects (all values of $F < 1$). Planned contrasts revealed significant effects of geometry for both attention-drawing procedures (each $t(15) > 5.7$, $P < 0.001$).

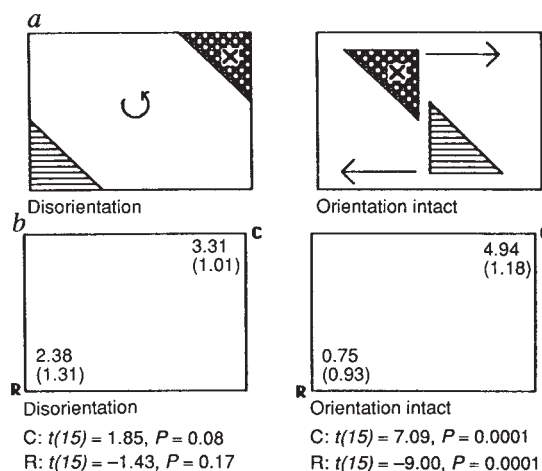


FIG. 4 Conditions and search patterns for the disorientation and orientation-intact tasks (each $n = 16$; mean ages 21.1 and 21.3 months respectively; 5 additional subjects failed to complete the experiment). *a*, Each child participated in one 4–6-trial search session in the white room with two triangular-solid containers of the same dimensions but different patterns (stripes or dots) and colours. The left diagram depicts the search environment throughout the disorientation task and at the end of each orientation-intact trial. The right diagram depicts the environment at the start of each orientation-intact trial. After the object was hidden, subjects' eyes were closed, facing the same direction as disoriented subjects at the end of disorientation, and the containers were moved to the corners as shown. *b*, Search patterns as before. The data from experiment 4, analysed using a $2(\text{sex}) \times 2(\text{condition}) \times 2(\text{location})$ ANOVA, revealed a significant interaction of condition and location ($F(1, 28) = 17.15$, $P < 0.001$) and no other significant effects (all P values > 0.10). Planned comparisons revealed significant effects of choosing $C_{(\text{orientation-intact})}$ more than $R_{(\text{orientation-intact})}$, of choosing $C_{(\text{orientation-intact})}$ more than $C_{(\text{disorientation})}$, and of choosing $R_{(\text{disorientation})}$ more than $R_{(\text{orientation-intact})}$ (all values of $t(15) > 4.2$, $P < 0.001$). In the disorientation condition, choice of C did not exceed choice of R ($P > 0.20$). A further study showed that children can remember and use non-geometric information to find an object after they are disoriented, provided the information does not serve as a cue to reorientation. Subjects were disoriented as in experiment 4, brought outside the testing room with the two containers, disoriented further, and asked to find the object; they reliably chose the container with the appropriate non-geometric markings.

accord with non-geometric landmarks that could have improved their performance, even though most children turned and inspected the room before searching and used the same landmarks in a different search task. These findings provide evidence for a common shape-based orientation mechanism in humans and other mammals and for informational encapsulation⁸ in the child's mechanism.

In contrast to young children and mature rats, human adults conjoined geometric and non-geometric information to reorient themselves. Their performance suggests that some representational systems become more accessible and flexible over development and evolution^{13,14}. Studies of the mechanisms underlying the increase in flexibility for reorientation may shed light on uniquely human capacities for problem solving. □

Received 25 October 1993; accepted 13 May 1994.

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ACKNOWLEDGEMENTS. We thank F. Keil, C. R. Gallistel, C. Williams, P. Bloom, R. Darlington and D. Simons for their comments, and L. Karavasilis and D. Kim for technical support.

An eye-specific G β subunit essential for termination of the phototransduction cascade

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HETEROTRIMERIC G proteins couple various receptors to intracellular effector molecules. Although the role of the G α subunit in effector activation, guanine nucleotide exchange and GTP hydrolysis has been well studied^{1–4}, the cellular functions of the G β subunits are less well understood^{5,6}. G $\beta\gamma$ dimers bind G α subunits and anchor them to the membrane for presentation to the receptor^{7–9}. In specific systems, the G β subunits have also been implicated in direct coupling to ion channels and to effector molecules^{10–19}. We have isolated *Drosophila melanogaster* mutants defective in an eye-specific G-protein β -subunit (G β e), and show here that the β -subunit is essential for G-protein–receptor coupling *in vivo*. Remarkably, G β mutants are also severely defective in the deactivation of the light response, demonstrating an essential role for the G β subunit in terminating the active state of this signalling cascade.

Genetic screening²⁰ for mutations in a photoreceptor-cell-specific G β subunit (G β e)²¹ isolated two alleles, G β e¹ and G β e². Both alleles have missense mutations which severely reduce the

levels of the G β subunit (Fig. 1a). G β e¹ has a tyrosine substituted for a cysteine at amino acid 293 and produces <0.5% of the wild-type levels of the G β subunit, whereas the G β e² allele has a glycine substituted for a glutamate at residue 288 and produces ~5% of wild-type protein levels.

When whole-cell patch clamp recordings^{22,23} were used to analyse the electrophysiological response of the G β e¹ and G β e² mutant photoreceptors to light stimuli, both G β alleles showed a dramatic loss of light sensitivity, G β e¹ mutants showing a reduction of nearly two orders of magnitude (Fig. 1b). The kinetics of their photoresponse also differed markedly from that of wild

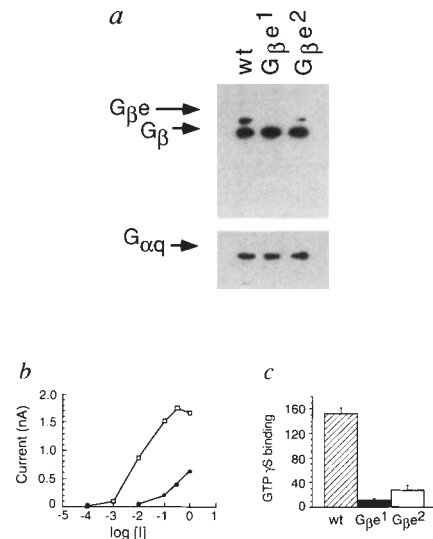


FIG. 1 a, Quantification of G β e protein levels. G β e¹ = 0.4% $\pm$ 0.2; G β e² = 4.6% $\pm$ 5.7 (range 0.8–16%). Similar results were obtained using two different antibodies with two different specificities. G β and G α q refer to a brain-specific isoform of G β ³² and to the photoreceptor-cell-specific G α ²⁷. As neither mutation affected the levels of G β e transcripts (data not shown), the dramatic reduction of G β e levels in these missense mutants is probably due to the synthesis of misfolded or unstable proteins that are rapidly degraded in the cell. Neither G β mutation affected the expression of a photoreceptor-cell-specific G α subunit, or of several other molecules involved in the phototransduction cascade (data not shown). b, Intensity response functions for wild-type (w¹¹¹⁸) and mutant photoreceptors. Note that 100-fold stronger light was required to evoke a minimal current from G β e¹ photoreceptors (closed circles) than from wild-type cells (open squares). Equivalent results were obtained from seven cells from each genotype. Photoreceptors were isolated and patch clamp recordings performed as previously described²². c, Blue-light-stimulated eye-specific GTP- γ S binding. Dark-adapted *Drosophila* head sections were stimulated with blue, 480-nm light (R $\rightarrow$ M conversion) or red, 610-nm light (M $\rightarrow$ R conversion). GTP- γ S binding to eye tissue was determined by autoradiography. In wild-type controls, GTP- γ [³⁵S] binds to target substrates in the compound eyes following blue light illumination which activates Rh1 rhodopsin. However, no retina-specific binding is observed following stimulation with red light (610 nm). G β e mutants showed dramatically reduced levels of blue-light-stimulated GTP- γ [³⁵S] eye-specific binding. Values shown indicate per cent of maximal wild-type response at saturating blue light intensity (w¹¹¹⁸, n = 9; G β e¹, n = 7; G β e², n = 7).

METHODS. Cryostat sections (10 μ m thick), each containing ~40 fly heads on nitrocellulose filters (Schleicher and Schull BA85, 25-mm circles), were pre-flashed for 10 s with red light (Schott RG-610 filter) in 50 mM 4-morpholine propanesulphonic acid, pH 6.7, 5 mM MgCl₂, 2 mM 2-mercaptoethanol, 2 μ g ml⁻¹ pepstatin, 10 μ g ml⁻¹ leupeptin, 2 mM benzamide, 0.2 mM ATP. Sections were then stimulated with red or blue (Schott BG-28) test flashes in the presence of 96 nM GTP- γ S and 4 nM GTP- γ [³⁵S]. After the light flashes, the filters were incubated in the dark for 1 min and washed with 10 mM sodium phosphate, pH 7.5, 150 mM NaCl, 0.1% Tween-20, then with 0.1 $\times$ SSC. Details of the procedure will be published elsewhere.

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type (Fig. 2). Whereas wild-type photoreceptors responded to intense light flashes with short latencies and short times to peak (27.3 ± 2.42 and 27.6 ± 10.8 ms, respectively^{22,24}) (Fig. 2a, f), in $G\beta^1$ mutants both parameters were dramatically increased (46.3 ± 5.99 and 103.6 ± 29 ms, respectively; $P < 0.0001$) (Fig. 2b, e, f). These slower response kinetics probably result from reduced levels of $G\alpha\beta\gamma$ holocomplexes available to couple to the downstream effectors. Interestingly, activation kinetics in the less severe $G\beta^2$ allele were only slightly altered (latency, 30.4 ± 1.67 ; time to peak response, 37.6 ± 8.17 ms; Fig. 2f). This shows that 5% of the wild-type levels of $G\beta$ polypeptide are sufficient for normal activation responses and is consistent with a catalytic role for $G\beta$ in the activation phase of the G-protein cycle. The changes in physiology of $G\beta$ mutants are due solely to the loss of $G\beta$, as reintroduction of the wild-type $G\beta^+$ gene into $G\beta^1$ mutant hosts by germ-line transformation fully rescued these defects (Fig. 2d, f).

Phototransduction in *Drosophila* occurs via a phospholipase-C-mediated signalling cascade^{25,26}. Light-activated rhodopsin interacts with a photoreceptor-cell-specific G_{aq} (ref. 27) which activates a phospholipase C effector. Biochemical evidence suggests that the reduced sensitivity of $G\beta$ mutants is due to a defect in the activation of G_{aq} . An *in-situ* assay of GTP- γ S binding to *Drosophila* eyes *in vivo* showed that light-induced GTP binding was dramatically reduced in $G\beta^1$ mutants (Fig. 1c). The magnitude of the loss paralleled the loss of sensitivity of the electrical response (Fig. 1a, and data not shown). These results suggest that the changes in light sensitivity, latency and time to peak in $G\beta$ mutants reflect the inability of the G_α subunit to couple efficiently to rhodopsin in the absence of $G\beta$ and demonstrate the essential requirement of $G\beta$ for G_α function *in vivo*.

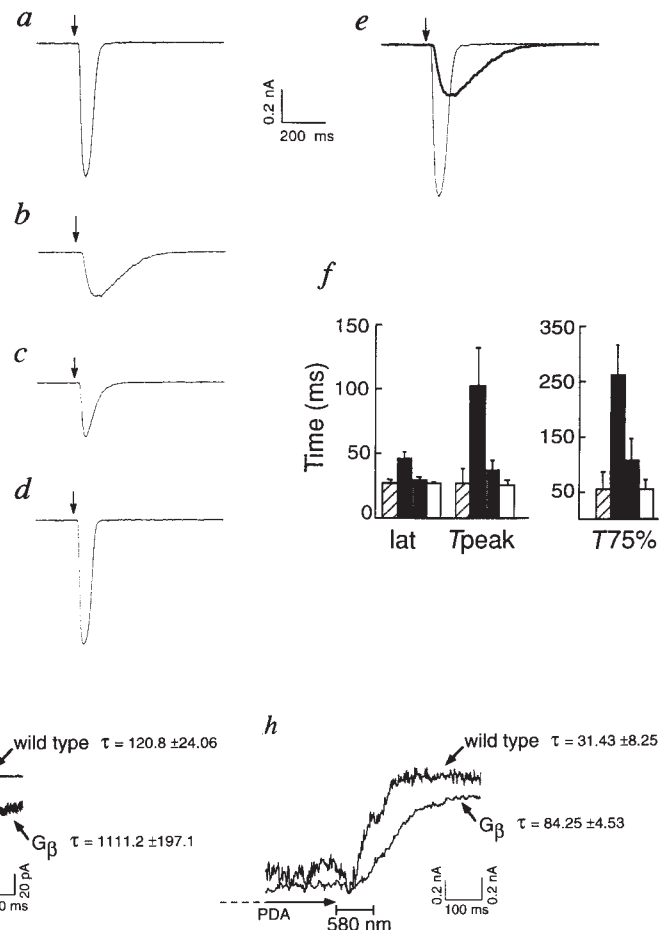
In addition to the expected defects in the activation phase of the light response (that is, coupling to the receptor molecule),

the rate of deactivation (defined as the recovery of the photoresponse) of the phototransduction cascade (Fig. 2e, f) was also dramatically and unexpectedly decreased in $G\beta$ mutants. To quantify the data, we measured deactivation as the time from peak response to 75% attenuation of the peak current (referred to as $T_{75\%}$). Wild-type photoreceptors had $T_{75\%}$ values of 55.3 ± 32.2 ms, compared with 261.6 ± 52.7 ms ($P < 0.0001$) in $G\beta^1$ mutants. These deactivation defects are indeed due to the loss of $G\beta$, as wild-type kinetics were restored after germ-line transformation with a cloned copy of the wild-type $G\beta$ gene (Fig. 2f). $G\beta^2$ mutants also showed defective deactivation kinetics ($T_{75\%} = 106.4 \pm 38.84$; $P < 0.05$; Fig. 2f), demonstrating that 5% of the wild-type levels of the protein are not sufficient for normal deactivation.

When light-activated channels open, extracellular calcium enters the photoreceptor cell, promoting termination of the light response^{22,23}. Thus, the altered deactivation kinetics of $G\beta$ mutants might be due to a decrease in calcium influx resulting from the reduced sensitivity of the $G\beta$ mutant photoreceptors. We analysed the electrophysiological response of wild-type and $G\beta$ mutants in the absence of extracellular calcium so as to eliminate the contribution of calcium-mediated deactivation mechanisms. Figure 2g shows that even in calcium-free recording medium, $G\beta^1$ photoreceptor deactivation kinetics are still markedly reduced compared with wild-type cells.

The $G\beta$ deactivation defects were examined further by analysing the kinetics of recovery of the photoresponse following continuous activation of the photoreceptor cell. This prolonged depolarizing afterpotential occurs whenever there is an excess of activated metarhodopsin over free arrestin²⁰. A prolonged depolarizing afterpotential can be experimentally terminated by photoconversion of metarhodopsin back to rhodopsin with 580-nm light²⁸. Because metarhodopsin in such a case is instantan-

FIG. 2 $G\beta$ mutants have defective activation and deactivation kinetics. Whole-cell voltage clamp recordings^{22,23} of light-activated currents from wild type (w^{1118}) (a), $G\beta^1$ (b), $G\beta^2$ (c) and $G\beta^1$; $P[w^+, G\beta^+]$ (d) transgenic flies. e, A superimposition of the wild-type and $G\beta^1$ responses on the same current and time scales. Arrows indicate the position of 10-ms light flashes of maximal light intensity ($\log [I] = 0$ in Fig. 1b). f, Histograms of latency, time to peak and time to 75% deactivation (mean $\pm$ s.d.; note the different time scales). Each data point represents 4–6 independent cells, each with a minimum of three individual electrophysiological recordings. Hatched bars, wild-type; solid bars, $G\beta^1$; shaded bars, $G\beta^2$; open bars, $G\beta^1$; $P[w^+, G\beta^+]$. lat, latency (time from stimulus onset to initiation of inward current); T_{peak} , time required to reach peak current from onset of stimulus; $T_{75\%}$, time from peak response to 75% attenuation of the peak current. g, Wild-type and $G\beta^1$ responses to 10-ms flashes in nominal calcium-free bath solution. For quantitative evaluation of the data, the tail of the deactivation phase of the light-activated current was fitted to a single exponential function and the time constant (τ) was measured (this is necessary owing to the extremely long deactivation times of $G\beta^1$ mutants in the absence of extracellular calcium). Average τ values (mean $\pm$ s.d.) are shown for wild type ($n = 7$) and $G\beta^1$ ($n = 4$). h, A prolonged depolarizing afterpotential (PDA) was induced in $G\beta^1$ and wild-type photoreceptors with a 100-ms pulse of 480-nm light (R $\rightarrow$ M conversion). The afterpotential was terminated 1.3 s later with a 100-ms pulse of 580-nm light (M $\rightarrow$ R conversion). The traces show the last 100 ms of the afterpotential and the deactivation phases following the 580-nm light pulse. Average τ values (mean $\pm$ s.d.) for wild type ($n = 4$) and $G\beta^1$ ($n = 3$) are indicated.



neously inactivated, it is possible to determine whether defects in receptor coupling or inactivation produce the slow kinetics of deactivation seen in the *Gβe* mutants. Upon light-induced inactivation of metarhodopsin, *Gβe* mutants still showed very slow deactivation kinetics (Fig. 2h), indicating that the process responsible for the deactivation defect in *Gβe* mutants lies downstream of receptor activation and inactivation.

Although much is known about G-protein function in reconstituted systems, less is known about the function of *Gβ* and *Gγ* *in vivo*. The demonstration that 5% of the wild-type levels of *Gβ*² in the weaker mutant 'rescues' the activation defects but does not rescue the deactivation phase of the response highlights the two aspects of *Gβ* requirements *in vivo*: first, there is a catalytic requirement in coupling, where *Gβ* probably functions by shuttling many *Gas* to the receptors, and second, there is a stoichiometric requirement where *Gβ* may need to complex with an activated component to shut off the response.

Termination of this signalling cascade may be modulated by *Gβ* at several steps. *Gβ* may act at the level of *Gα* or the *Gα*:effector complexes²⁹. It may also be required at a different point in the signalling pathway. Pitcher *et al.*³⁰ showed that *Gβγ* is necessary for phosphorylation of the *β*-adrenergic receptor by recruiting the *β*-adrenergic receptor kinase to the membrane. However, as the deactivation defect of *Gβe* mutants cannot be rescued following photochemical inactivation of rhodopsin (Fig. 2h), *Gβ* is not required at the level of the receptor molecule. Ron *et al.*³¹ showed that homologues of *Gβ* subunits may function as binding sites for protein kinase C. Interestingly, *Drosophila* mutants defective in an eye-specific isoform of protein kinase *Ca* have deactivation defects similar to *Gβe* mutants^{22,24}. The strict requirement for *Gβ* in modulating the shut-off of the response may be particularly important in signalling cascades like phototransduction, where *Gβγ* molecules are very abundant and fast deactivation kinetics are fundamental for proper temporal resolution of closely spaced stimuli. □

A matrix metalloproteinase expressed on the surface of invasive tumour cells

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GELATINASE A (type-IV collagenase; *M_r* 72,000) is produced by tumour stroma cells and is believed to be crucial for their invasion and metastasis, acting by degrading extracellular matrix macromolecules such as type IV collagen¹⁻³. An inactive precursor of gelatinase A (pro-gelatinase A) is secreted and activated in invasive tumour tissue⁴⁻⁷ as a result of proteolysis which is mediated by a fraction of tumour cell membrane that is sensitive to metalloproteinase inhibitors^{4,5}. Here we report the cloning of the complementary DNA encoding a new matrix metalloproteinase with a potential transmembrane domain. Expression of the gene product on the cell surface induces specific activation of pro-gelatinase A *in vitro* and enhances cellular invasion of the reconstituted basement membrane. Tumour cells of invasive lung carcinomas, which contain activated forms of gelatinase A, were found to express the transcript and the gene product. The new metalloproteinase may thus trigger invasion by tumour cells by activating pro-gelatinase A on the tumour cell surface.

We screened cDNAs with homology to conserved regions in matrix metalloproteinase (MMP) genes (Fig. 1 legend) and isolated a unique 3.4-kilobase (kb) cDNA fragment (MMP-X1) from a human placenta cDNA library. This cDNA encodes a unique protein of 582 amino acids (*M_r* 66K) which can be closely aligned with known MMP family members^{8,9} (Fig. 1a, b). The MMP-X1 protein contains a potential transmembrane domain at the C terminus (24 hydrophobic amino acids; black overbar in Fig. 1a), which does not exist in other MMPs. We will call the product MT-MMP, for membrane-type matrix metalloproteinase.

To identify the gene product, we transfected COS-1 cells with MT-MMP plasmid and immunoprecipitated cell lysates labelled with ³⁵S-methionine with monoclonal antibodies (antibodies 113-5B7, 114-1F2 and 118-3B1) raised against synthetic MT-MMP peptides. These antibodies recognized different epitopes but immunoprecipitated a common 63K protein only from the transfected cells (Fig. 2a). We could detect no MT-MMP released into the medium, whereas a tissue inhibitor of metalloproteinases type-1 (TIMP-1), which was co-expressed as a secretory protein, was found in both the cell lysate and culture medium (Fig. 2b). MT-MMP was also detected in the plasma membrane fraction of the transfected cells (Fig. 2c) but TIMP-1 was not (data not shown). Immunohistochemical staining of COS-1 cells transfected with MT-MMP plasmid localized the product to the cell surface (Fig. 2d).

The expression of MT-MMP on the cell surface fits the requirements of an activator for pro-gelatinase A²⁻⁷. We tested this idea by transfecting MT-MMP plasmid into human fibrosarcoma HT1080 and mouse fibroblast NIH3T3 cell lines which secrete pro-gelatinase A and pro-gelatinase B (92K type-IV procollagenase) into the culture supernatant^{10,11}, as detected by gelatin zymography (66K and 90K bands, respectively, in Fig. 3a).

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Received 5 January; accepted 18 May 1994.

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ACKNOWLEDGEMENTS. We thank D. Lindsley, E. Ross, R. Tsein and members of the Zuker laboratory for discussion; G. Froelich for sectioning fly heads for biochemical analysis; and R. Hardy for help in the genetic screen. P.J.D. was supported by a National Eye Institute (NEI) postdoctoral fellowship. H.M.-S.-H. was supported by an American Cancer Society Junior fellowship and N.J.C. by a grant from NEI. C.S.Z. and J.H. are investigators of the Howard Hughes Medical Institute.

FIG. 1 The MT-MMP protein sequence encoded by MMP-X1 cDNA. MMP-X1 cDNA has a large open reading frame between nucleotides 112–1,857 which encodes a protein of 582 amino acids (GSDB/DBJ/EMBL/NCBI accession number D26512). a, Alignment of amino-acid sequences of MMPs^{9,9}. MT, MT-MMP (MMP-X1 protein; see text); ST3, stromelysin-3 (ref. 21); ST1, stromelysin-1 (ref. 24); COL, interstitial collagenase²⁵; 72K, gelatinase A¹⁵; 92K, gelatinase B²⁶. Conserved amino acids are boxed. The putative functional domains of MT-MMP were assigned as follows (see b): a signal peptide with a hydrophobic core sequence (20 N-terminal amino acids), following a propeptide domain of sequence PRCGVPD (dots above)²⁷; a unique insertion of 10 amino acids similar to stromelysin-3 (potential recognition sequences for furin-like enzymes²⁸; underlined RXKR conserved between them); the core enzyme domain showing the highest conservation with a potential zinc-binding site²⁹ (shaded overbar); a hinge domain (285–318) and a potential transmembrane domain (black overbar top) in a haemopexin-like domain (367–582). b, Diagram of the MT-MMP protein. METHODS. cDNA fragments having homology to MMP genes were amplified from human placenta cDNA using degenerated polymerase-chain reaction (PCR) primers corresponding to the conserved amino-acid sequences in the cysteine switch (PRCGVPD) and in the catalytic domain (GDHFDXXE) (manuscript in preparation). Isolated fragments were sequenced and one with a unique sequence was used as a probe to isolate a longer cDNA from the human placenta cDNA library.

a

MT	MSPAPRPSRCIL-LPL-LTLG-----TALAS	LGSAQSSSPSEAWLQYGYLFPGLDRLTET	QRSQSLSAIAAMQKFFGLQVTKADADT	84
ST3	MA---P---AAWL-----RSA-----AARAL	LPPMLLLLPPLPPLARALPF--DVHLL--	HAERGPQPQWHAALPSSPAPAPATQEAAP	71
ST1	MKSL-PI-LLLLCAVACSAYP-----LDGAA	RGEDTSMNLVQKYLENYVDLKK-DVKQFVR	RKDSGPVVKIKRMQKFFGLQVTKADADT	83
COL	MBSFPL-LLLLFWGV-VSHS-----FPATL	ETQEQVDLVQKYLENYVDLKK-DGRQVEK	RKDSGPVVKIKRMQKFFGLQVTKADADT	83
72K	-----AP-----SPIIK	FPQDVAPK-TDKELAVQYLYNTF-YGCKPE-	SCNLFVLKDTLKKMKQFFGLQVTKADADT	64
92K	MSLWQPLVLLVLLGCCFAAPRQSTLV	FPGLDRLNLTDRQLAEYLYRYGYTRVAM	RGEKSLGALLLQKQLSLPTEGELDSAT	90

MT	MKAMRPRCGVPD-KGAEIKANVRKRYAI	Q-G-LMMHNEITFCITNYTFKVGAYATY	ALRMAFVRESNTPHFEVVPYAYIREGHE	172
ST3	ASSLRPRCGVPD-PSDGLSARNRQKRFVL	S-G-GWKEKTDLYNLLRFPWQLVQEQVRQ	TMARALKVSDVTPHFEVVPYAYIREGHE	150
ST1	LEVNRPRCGVPD-VGH-----FRT	FPGLPWRKTELTYNLLRFPWQLVQEQVRQ	AVEHALKVEVTPHFEVVPYAYIREGHE	154
COL	LKVMKPRCGVPD-VAQ-----FVL	TEGNPRWQTELTNYNLLRFPWQLVQEQVRQ	AIENAFOLWNTVTPHFEVVPYAYIREGHE	154
72K	IETMRPRCGVPD-VAN-----YNT	FPKPRWQKNTTYNLLRFPWQLVQEQVRQ	AFANAFOLWNTVTPHFEVVPYAYIREGHE	135
92K	LKAMRPRCGVPD-LGR-----FQT	FEGLLWHEHNTTYNLLRFPWQLVQEQVRQ	AFANAFOLWNTVTPHFEVVPYAYIREGHE	161

MT	KQADIITFAEGFHCDRTFDCGGHLLABA	YFSPNIGDHFDAEHTVVRNEDLN---	229
ST3	GRADILITARYWDCDLHFDGCGHLLABA	YFSPNIGDHFDAEHTVVRNEDLN---	207
ST1	GEADILITARYWDCDLHFDGCGHLLABA	YFSPNIGDHFDAEHTVVRNEDLN---	211
COL	QADILITARYWDCDLHFDGCGHLLABA	YFSPNIGDHFDAEHTVVRNEDLN---	211
72K	GEADILITARYWDCDLHFDGCGHLLABA	YFSPNIGDHFDAEHTVVRNEDLN---	225
92K	RDADILITARYWDCDLHFDGCGHLLABA	YFSPNIGDHFDAEHTVVRNEDLN---	251

MT	-----	-----	-----	229
ST3	-----	-----	-----	207
ST1	-----	-----	-----	211
COL	-----	-----	-----	211
72K	GLFWCSTTYNFEKDGKYGFCPEHALFTNGG	NAEQGQCKFPFRQGTSDYSCITEGRDGY	RWCSTTGYDRDKKYGFCPEHALFTNGG	314
92K	GLFWCSTTYNFEKDGKYGFCPEHALFTNGG	NADGKPCQFPFRQGTSDYSCITEGRDGY	RWCSTTGYDRDKKYGFCPEHALFTNGG	341

MT	-----	-----	-----	268
ST3	-----	-----	-----	242
ST1	-----	-----	-----	248
COL	-----	-----	-----	245
72K	SEGAFCVPTFTFLGNKYSCTSGRSDGKM	WCATTANYDDRRKMGFCPDQGYSLFVARE	EGEANGLESSTDPGALMPTIYTY--TKN	401
92K	SAGELCVPTFTFLGNKYSCTSGRSDGRL	WCATTANYDDRRKMGFCPDQGYSLFVARE	EGEANGLESSTDPGALMPTIYTY--TEG	428

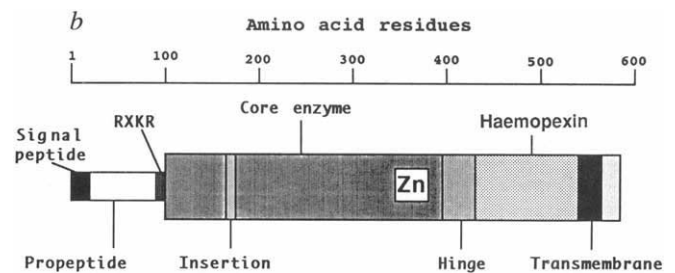
MT	FVLPEDPDKGQVHLYG-----	-----	-----	316
ST3	LELEPDNDKGVHLYG-----	-----	-----	291
ST1	FVLEPDNDKGVHLYG-----	-----	-----	287
COL	VLEPDNDKGVHLYG-----	-----	-----	275
72K	FVLEPDNDKGVHLYG-----	-----	-----	437
92K	FVLEPDNDKGVHLYG-----	-----	-----	513

MT	NICDGN--FDTVAMLRGEMFCKRWRVVR	RNNQVMDGYMP--IGQFMRGLPASINTAY	ERK-DGKVFVHCKDKEHVTDEASLEHMRK	401
ST3	DACE-A-SFDAVSTIRGELFFKAGFVWRL	RGQ--LQPGYPALASRHWQLSPVDAAFP	EDA-QGHVWFPCQVWYVDGEKPVLGPA	375
ST1	ANCPALSPDAVSTIRGELFFKAGFVWRL	SLRK--LEPELS-LISSFVSLSPGVDAAF	EUTSKDLVFPQVWYVDGEKPVLGPA	374
COL	KACDKLTPDAITIRGEVHFFKDRFYHRT	NPFY--PEVELN-FTSVTFQLNGLAAY	EFADREVEVHFFKDRFYHRTVQGVLEMRK	362
72K	EICKQDIPTDIAIRGEVHFFKDRFYHRT	VTPR--DKPHGPLLVAHFFKDRFYHRT	EFADREVEVHFFKDRFYHRTVQGVLEMRK	525
92K	DACVNI-GLDAIAIGNQLYLPKDKYVVR	SEGRSRPQGF--LIADKWPALFKLDSVF	DEPLSKKLFHFFKDRFYHRTVQGVLEMRK	599

MT	BIKELGRGLPTD--KIDNALFWNPG-KTY	FERGNKYRFEFNEELRAVDSEYFKNI-KVWE	GIPESPRGFMGSDEVT--TYFYKGNKYWF	486
ST3	PLTEL--GLVRFP--VHVALVWVGEKNIY	FERGRDYRFPSPSTRVDSVPVPR-ATDWR	GVFSEIDAA--QDADGY-HYFRLGRLYWF	458
ST1	GIBTEL--GPPPTVRKIDNALSDK-ZKNKY	FPVZDKYRFPDEKRSMDPGFPKQIAEDFP	GIDSKIDAVF--MKDGF-HYFRLGRLYWF	458
COL	DIYSS--GPPPTVRKIDNALSDK-ZKNKY	FPVANKYRFPDEKRSMDPGFPKQIAEDFP	GIGKIDAVF--MKDGF-HYFRLGRLYWF	447
72K	PLTSL--GLPPOVDVDAFNFWSKX-KTY	IFAGDKFVRFNEVKKMDPGFPKLIADAWN	AIPDNLDVVDLQGGGH--SYFFKQAYLYKL	611
92K	RLDEL--GLGADVAQVTCALRSRG-KML	IFSGRLRFPDVKAMVDPRSASEVDRMFP	GVPLDTHDFV--QYREKAYTCQDRFYWRV	682

MT	NNQKLKVEPGYKPSALRDWNGCPSGGRDPE	GTEETEVIIEVDDEGGGVAASAAVLEVP	LLLLVLAVGLAVFFRRRGTPRRLLYCQR	576
ST3	DPVKKVLEPGYKPSALRDWNGCPSGGRDPE	-----	-----	482
ST1	DPNAKVTET--LKNS-----	-----	-----	477
COL	DPNKKRLTLQ--KANS-----	-----	-----	469
72K	ENQSLKSVKPGSIR--SD-----	-----	-----	631
92K	SSRSZLNQVDQGVVYTD-----	-----	-----	706

MT	SLLDKV--	-----	-----	582
ST3	PANTFL--	-----	-----	488
ST1	-----	-----	-----	477
COL	N-----	-----	-----	469
72K	-----	-----	-----	631
92K	D-----	-----	-----	707



Expression of MT-MMP (confirmed as in Fig. 2a; data not shown) generated two new gelatinolytic bands of 64K and 62K (Fig. 2a), corresponding to processed gelatinase A fragments precipitated with anti-gelatinase A antibody (Fig. 3c); pro-gelatinase B was unaffected. Migration of these fragments was the same for activated forms of gelatinase A from HT1080 cells treated with concanavalin A^{4,10-14} (Fig. 3a), in which MT-MMP messenger RNA expression was also stimulated (data not shown). Insect Sf9 cells infected with recombinant baculovirus harbouring MT-MMP cDNA expressed no soluble gelatinolytic activity (Figs 2a and 3b), so we used these cells to examine the effect of MT-MMP on exogenous pro-gelatinase A. When Sf9 cells were incubated with HT1080-cell-conditioned medium, pro-

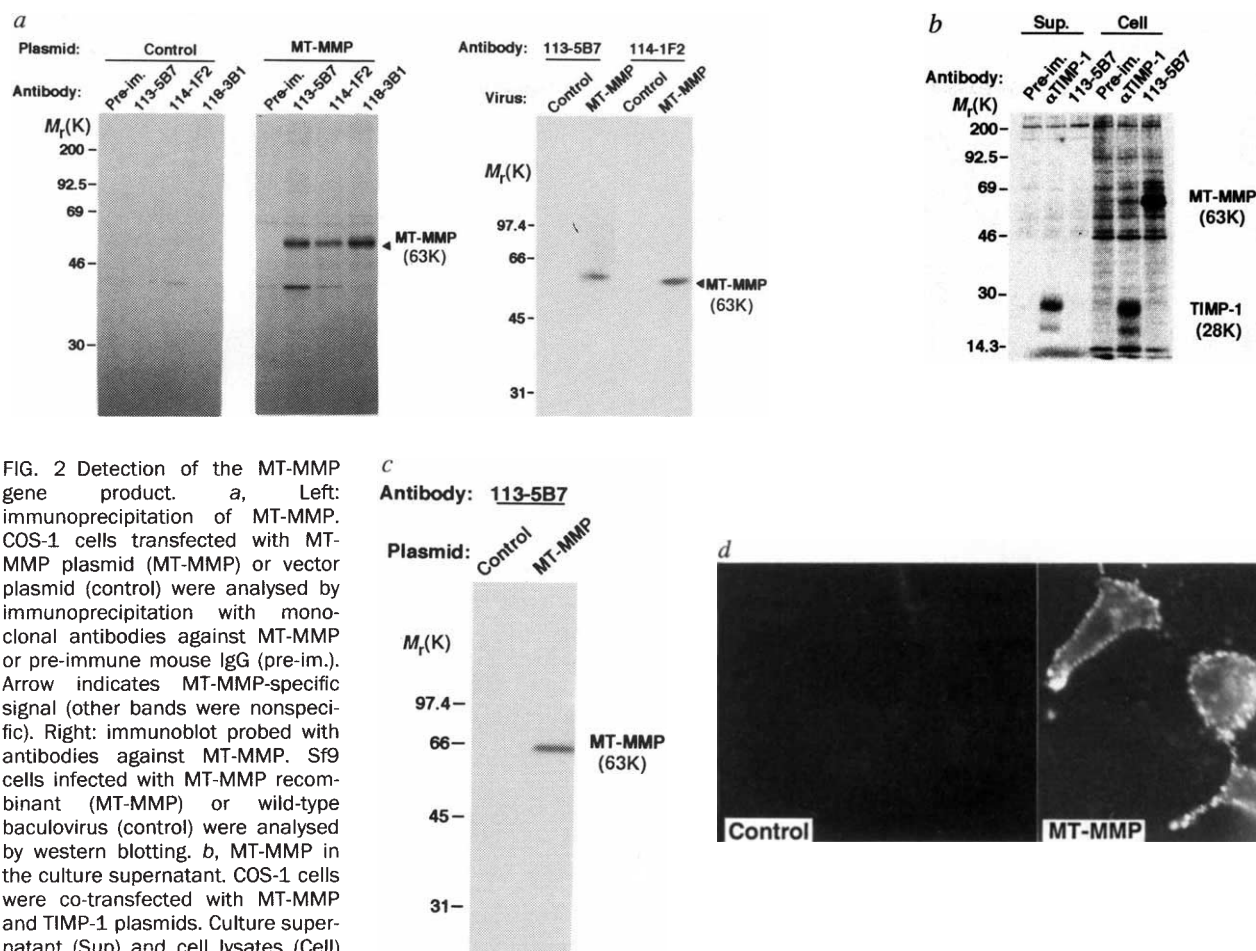


FIG. 2 Detection of the MT-MMP gene product. **a**, Left: immunoprecipitation of MT-MMP. COS-1 cells transfected with MT-MMP plasmid (MT-MMP) or vector plasmid (control) were analysed by immunoprecipitation with monoclonal antibodies against MT-MMP or pre-immune mouse IgG (pre-im.). Arrow indicates MT-MMP-specific signal (other bands were nonspecific). Right: immunoblot probed with antibodies against MT-MMP. Sf9 cells infected with MT-MMP recombinant (MT-MMP) or wild-type baculovirus (control) were analysed by western blotting. **b**, MT-MMP in the culture supernatant. COS-1 cells were co-transfected with MT-MMP and TIMP-1 plasmids. Culture supernatant (Sup) and cell lysates (Cell) were immunoprecipitated with antibodies against MT-MMP (113-5B7) and TIMP-1 (α TIMP-1), and with pre-immune IgG (Pre-im.). **c**, Immunoblot of plasma membrane preparations. Samples were prepared from the COS-1 cells transfected with MT-MMP (MT-MMP) or vector (control) plasmid. **d**, Immunostaining. COS-1 cells transfected with MT-MMP (MT-MMP) or vector plasmid (control) were stained with an antibody (113-5B7) without fixation. **METHODS**. Mouse antibodies 113-5B7, 114-1F2 and 118-3B1 were raised against peptides CDGNFDTVAMLRGEM (residues 310–333), REVYAYIREGHEK (160–173) and PKSALRDWMGCPSSG (498–512), respectively. The MT-MMP gene was expressed using a eukaryotic expression vector pSG5 (Stratagene). For immunoprecipitation, 35 S-

methionine-labelled protein samples were incubated with antibodies, antigen–antibody complexes were collected and resolved by electrophoresis on a 10% SDS–polyacrylamide gel, and analysed by a Bio-image analyser BA100 (Fuji Photo Film Co.). Immunoblots were visualized by enhanced chemiluminescence (Amersham). The plasma membrane fraction was prepared by a discontinuous sucrose gradient centrifugation after disruption of cells in a Dounce homogenizer¹⁴. Immunohistochemistry of the cells was done without fixation using antibody 113-5B7 and goat anti-mouse IgG antibody conjugated with fluorescein isothiocyanate (FITC).

gelatinase A became activated (Fig. 3b). Gelatinolytic activity in culture medium was also activated upon co-transfection of COS-1 cells with pro-gelatinase A and MT-MMP plasmids (data not shown); this activity was co-localized with MT-MMP in the plasma membrane fraction of the cells (Figs 2c and 3d) and was blocked by recombinant TIMP-2, a specific inhibitor of MMPs (data not shown).

The appearance of activated gelatinase A is a feature of many types of malignant tumour^{6,7}. We therefore investigated whether there was a correlation between the activation of pro-gelatinase A and MT-MMP expression in tumour tissues. Zymogram assay of lung carcinoma tissues demonstrated tumour-specific activation of pro-gelatinase A (Fig. 3e). Northern blot analysis of samples showed that there was more MT-MMP mRNA (4.5 kb) in the tumours than in normal lung tissue (Fig. 3e). MT-MMP was found by immunohistochemical analysis in the carcinoma cells (Fig. 3f) but not in the parenchymal or stromal cells of the surrounding normal tissue (data not shown).

As gelatinase A degrades various extracellular matrix macro-

molecules, including type IV collagen and laminin^{15,16}, we used the modified Boyden Chamber method¹⁷ to test whether MT-MMP expression enhanced the invasiveness of cells producing pro-gelatinase A into the reconstituted basement membrane (Matrigel). We found that MT-MMP expression in HT1080 and NIH3T3 cells increased the number of invasive cells more than 2-fold compared with controls and that the invasion was sensitive to TIMP-2 (Fig. 4). MT-MMP, however, did not affect chemotactic activity of the cells.

MMPs expressed at tumour sites are implicated in tumour invasion^{1,3,18,20}, but some of them may be produced by the stromal cells surrounding tumours rather than by tumour cells themselves^{2,3,21}. Pro-gelatinase A is mainly produced by the tumour stromal cells^{2,3,22} and can be activated on the tumour-cell surface^{4,23}. Thus, MT-MMP at the cell surface may link pro-gelatinase A production by stroma cells and invasion by tumour cells in response to activation of pro-gelatinase A at the invasive edge of tumour-cell nests. The putative transmembrane domain of MT-MMP is essential for this function, because when trun-

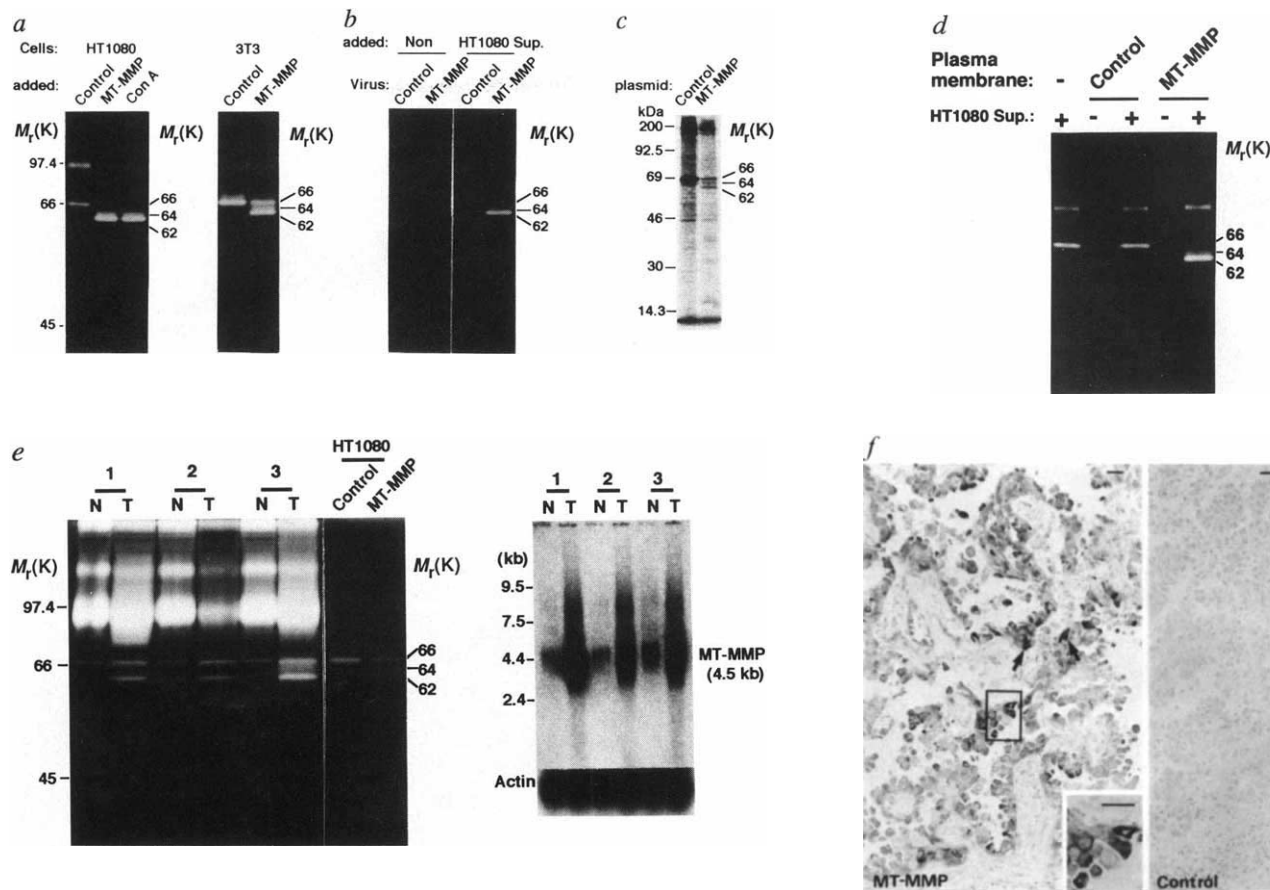
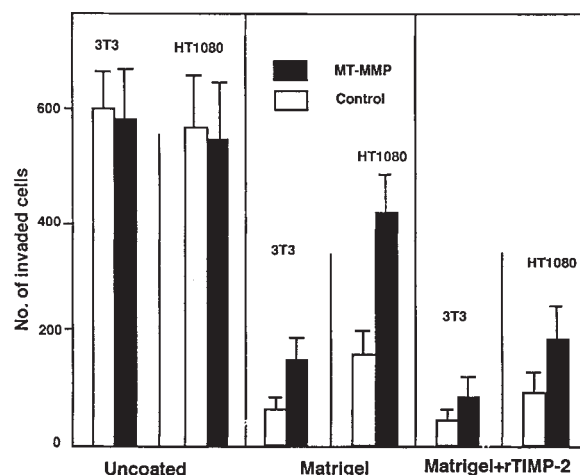


FIG. 3 Processing of pro-gelatinase A by MT-MMP expression. *a*, Gelatin zymography of conditioned medium of HT1080 and NIH3T3 cells. Cells were transfected with MT-MMP (MT-MMP), vector plasmid (control), or treated with 100 $\mu\text{g ml}^{-1}$ concanavalin A (Con A). *b*, Gelatin zymography of conditioned medium of Sf9 cells. Sf9 cells infected with recombinant (MT-MMP) or wild-type virus (control) were cultured in the presence (HT1080 Sup.) or absence of HT1080 conditioned medium (Non). *c*, Immunoprecipitation of gelatinase A. Conditional medium of HT1080 cells transfected with MT-MMP or vector plasmid were subjected to immunoprecipitation using anti-gelatinase A antibody. *d*, Gelatin zymography of plasma membrane preparations. Plasma membrane fractions of COS-1 cells transfected with MT-MMP (MT-MMP) or vector (Control) plasmid were incubated with (+) or without (-) conditioned medium from HT1080 cells. *e*, MT-MMP expression in lung carcinomas. Tumour (T) and corresponding normal (N) tissues of two lung adenocarcinomas and a squamous cell carcinoma were analysed by gelatin zymography (right) and northern blotting (left). *f*, Immunostaining of a human lung adenocarcinoma. MT-MMP, antibody 113-5B7; control, non-immune mouse IgG. Arrows indicate intense staining. Inset, high-power view of the enclosed area. Scale bar, 25 mm.

METHODS. Gelatin zymography was performed as described³⁰. Conditioned medium was prepared by cultivating cells in serum-free medium for 24 h. Gelatinase A activation by the plasma membrane fraction was achieved by incubating the fraction (20 μg protein) with the conditioned medium from HT1080 cells for 2 h at 37 °C. Immunoprecipitation is described in Fig. 2 legend. For northern blotting, total RNA was extracted from tissues and the blot probed with MT-MMP cDNA labelled with ³²P as described¹⁷. The blot was then reprobed with actin cDNA. Immunostaining with anti-MT-MMP mAb (113-5B7) was done on the fixed sections of a human lung adenocarcinoma and detected using an avidin-biotin complex.

FIG. 4 Expression of MT-MMP stimulates invasion of cells *in vitro*. NIH3T3 and HT1080 cells transfected with MT-MMP plasmid (MT-MMP) or control plasmid were assayed for invasion of the reconstituted basement membrane in the presence (Matrigel+rTIMP-2) or absence of 10 $\mu\text{g ml}^{-1}$ recombinant TIMP-2 (Matrigel). Motility was analysed on uncoated filters (Uncoated). METHODS. Invasion was assayed by a modified Boyden Chamber method¹⁷ according to the manufacturer's instructions (Becton Dickinson). HT1080 or NIH3T3 cells transfected with MT-MMP or control plasmid were suspended in DMEM medium containing 0.1% BSA and seeded onto an uncoated filter (8-mm pore size) or onto Matrigel-coated filters in Biocoat Matrigel invasion chambers. After 24 h incubation, filters were fixed with methanol and stained with a haematoxylin and eosin. Cells that invaded the lower surface of the filters were counted under a microscope at a magnification of $\times 400$. Each assay was done in triplicate.



cated its ability to activate pro-gelatinase A is abolished (manuscript in preparation). □

Received 31 January; accepted 17 May 1994.

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ACKNOWLEDGEMENTS. We thank M. Kita for technical assistance, Y. Watanabe and M. Tokuraku for surgical specimens, and S. Aratake for peptide synthesis. This work was supported in part by Special Coordination Fund for Promoting Science and Technology from the Ministry of Science and Technology of Japan, and by a grant-in-aid for Cancer Research from the Ministry of Education, Science and Culture of Japan, and by the Vehicle Racing Commemorative Foundation.

Disruption of *c-mos* causes parthenogenetic development of unfertilized mouse eggs

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THE *c-mos* proto-oncogene encodes a 37–39K cytoplasmic serine/threonine kinase¹ implicated in the meiotic maturation events during murine spermatogenesis² and oogenesis^{3–6}. In *Xenopus*, ectopic expression of pp39^{mos} can promote both the meiotic maturation of oocytes^{7–9} and also arrest the cleavage of blastomeres¹⁰. To elucidate the role of pp39^{mos} we have generated homozygous mutant mice by gene targeting in embryonic stem cells¹¹. These mice are viable and mutant males are fertile, demonstrating that pp39^{mos} is not essential for spermatogenesis. In contrast, mutant females, have a reduced fertility because of the failure of mature eggs to arrest during meiosis. *c-mos*^{−/−} oocytes undergo germinal vesicle breakdown and extrusion of both polar bodies followed in some cases by progression into cleavage. Mutant females also develop ovarian cysts. These results demonstrate that a major role for pp39^{mos} is to prevent the spontaneous parthenogenetic activation of unfertilized eggs.

The targeting vector, pMS1(neo)tk (Fig. 1a) contains a neomycin-resistance (*neo*^r) gene inserted into the middle of the single *c-mos* coding exon. This insertion introduces an amber stop codon into the *c-mos* reading frame to terminate translation upstream of sequences essential for kinase activity^{12,13}. pMS1(neo)tk was electroporated into CCE embryonic stem (ES) cells and clones resistant to both G418 and gancyclovir were screened for a targeting event. The expected genomic structure of the targeted *c-mos* allele was confirmed by Southern blotting using an external downstream genomic probe not present in the targeting construct. *Xba*I digestion, which does not cut within the *neo*^r gene, generated a fragment 1.8 kilobases (kb) larger than the wild-type fragment, a size increase consistent with insertion of the *neo*^r gene (Fig. 1b). *Xba*I–*Bam*HI double digestion (Fig. 1b) and *Pvu*II digestion (data not shown) confirmed that the insert within the *c-mos* coding exon was the *neo*^r gene.

Eight independently targeted clones were identified from 186 colonies and two were injected into C57Bl/6 blastocysts to give chimaeric animals. Seven out of 24 chimaeric males transmitted the ES cell genome to their progeny. Mice heterozygous for the disrupted *c-mos* allele (*mos*^{+/−}) were crossed to obtain mice homozygous for the mutation (*mos*^{−/−}) (Fig. 1b). Viable homozygous mutant mice were obtained at the expected frequency indicating that an intact *c-mos* gene is not required for normal embryonic development. Polymerase chain reaction (PCR) across the single *c-mos* coding exon using two different pairs of primers detected the presence of a wild-type exon only in mice with a wild-type allele and not in *mos*^{−/−} mice. Southern blot with a *mos* coding exon probe showed no intact wild-type *c-mos* exon in *mos*^{−/−} mice (Fig. 1b). Thus, the disrupted *c-mos* locus shows a genomic structure entirely consistent with the expected homologous replacement event.

Northern blot analysis of RNA extracted from *mos*^{+/+} ovaries and hybridized with *c-mos* exon fragments either upstream or downstream of the insert detected a clear band of messenger RNA of about 1.4 kb. There was no equivalent signal from equal amounts of RNA from *mos*^{−/−} ovaries suggesting that the insert may impair transcription or destabilize the transcript.

As pp39^{mos} has been implicated in both male and female gametogenesis we tested *mos*^{−/−} animals of both sexes for fertility. Homozygous mutant males sired offspring at comparable rates to control aged-matched litter mates (either *mos*^{+/+} or *mos*^{+/−}) indicating that an intact *c-mos* gene is not required for spermatogenesis. Histological examination of testes from null mutant animals also failed to identify gross differences from wild-type and showed the presence of mature sperm. In contrast, female mutant animals exhibited a reduced fecundity and gave rise to statistically significant small litters ($P < 0.005$). The average litter size from *mos*^{−/−} females was 2 ± 0.63 (7 breeding pairs, 12 offspring) whereas from a combined group of *mos*^{+/+} and *mos*^{+/−} females it was 7 ± 1.86 (12 pairs, 85 offspring). Such small litters were often cannibalized by the mother shortly after birth.

Because ectopic expression of pp39^{mos} can induce meiotic maturation of *Xenopus* eggs it was possible that the small litter sizes were a consequence of the failure of some eggs to mature in response to hormonal signals. In mice, the final stages of egg maturation are initiated in response to a surge of luteinizing hormone (LH) in the bloodstream. After LH stimulation, oocytes undergo germinal vesicle breakdown (GVBD) and initiate meiotic division leading to first polar body extrusion. At this point, eggs are released from the ovary ready for fertilization. To study these initial events in *mos*^{−/−} animals, eggs were

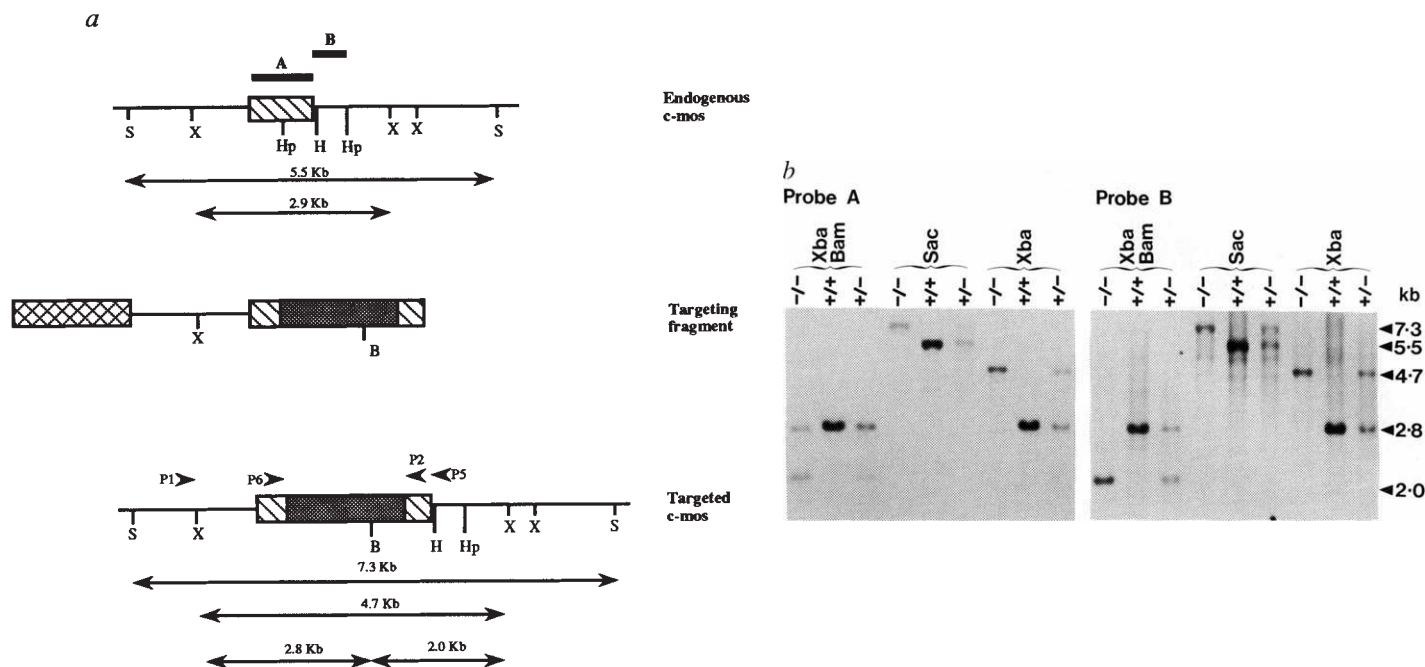


FIG. 1 Targeted disruption of the murine *c-mos* gene. *a*, Targeting vector and recombination at the *c-mos* locus. The *c-mos* 1-kb coding exon is shown hatched, the *neo*^r cassette stippled and the herpes simplex virus (HSV) thymidine kinase gene cross hatched. Bars marked A and B show the location of probes used to confirm targeting. PCR screening primers are shown by arrowheads. Expected restriction fragment sizes are indicated by arrows. B, *Bam*HI; H, *Hind*III; Hp, *Hpa*I; S, *Sac*I; X, *Xba*I. *b*, Southern blot analysis of the three genotype categories derived from heterozygote crosses.

METHODS. To generate the targeting plasmid, a 2.8 kb *Sac*I–*Hind*III *c-mos* genomic fragment from pMS-1²⁰ was cloned into the corresponding sites of pSP64 to give pSP(MS-1). A PGKneo cassette²¹ from pKJ-1

was released by *Eco*RI–*Hind*III digestion and blunt ligated into the unique *Hpa*I site within the *c-mos* coding region of pSP(MS-1) to generate pMS1(neo). A 2.0 kb blunt-ended *tk*-fragment driven by a modified HSV promoter obtained from pMC1neo was subcloned into the *Sac*I site of pMS1(neo) to generate the targeting vector pMS1(neo)tk. ES cells were cultured and electroporated with 5 µg linearized targeting construct as described previously²². G418^r/ganc^r clones were screened for a targeting event using a 500 bp *Bgl*II–*Hpa*I fragment (probe B) immediately downstream of the *c-mos* coding sequence. Lack of an intact *c-mos* coding exon was confirmed using a 900 bp *Ava*I–*Hind*III coding fragment (probe A).

isolated from Graafian follicles and matured *in vitro*¹⁴. Both mutant and wild-type eggs initiated GVBD and extruded the 1st polar body (Fig. 2) indicating that wild-type pp39^{mos} is not essential for normal oocyte maturation. This is in contrast to one of the functions of *c-mos* in *Xenopus* where ectopic expression promotes egg GVBD. As expected, eggs from wild-type animals arrested after formation of the first polar body (Fig. 2). However, *mos*^{−/−} eggs failed to arrest at this stage and proceeded to extrude the second polar body, consistent with parthenogenetic activation.

During maturation, *Xenopus* eggs accumulate pp39^{mos} which is rapidly degraded by the calcium-dependent cystein protease

calpain after fertilization¹⁵. Microinjection of synthetic *mos* RNA arrests the mitotic division of *Xenopus* blastomeres¹⁰, suggesting that one possible function of pp39^{mos} is as a component of the cytotstatic factor (CSF), which holds unfertilized eggs at meiotic metaphase II. Degradation of pp39^{mos} after fertilization would allow completion of meiosis. But degradation of pp39^{mos} may not be involved in the release from metaphase arrest because the kinetics of pp39^{mos} destruction are not consistent with this^{16,17}. Also, microinjection of antisense *c-mos* oligonucleotides into murine oocytes results in chromosome decondensation and reformation of a nucleus after meiosis I, suggesting that pp39^{mos} is required for meiosis II (ref. 6). To resolve the biological func-

FIG. 2 Parthenogenetic activation of *mos*^{−/−} eggs. Animals were injected intraperitoneally with 5 IU of PMS followed 48 h later by 5 IU of hCG. Representative photomicrographs of eggs at the times indicated (in h) after hCG administration (time zero). Polar bodies are indicated by arrowheads, pronuclei by arrows. Eggs isolated from Graafian follicles; 0 h, showing germinal vesicle; 12 h, germinal vesicle breakdown has occurred and first polar body extrusion; 24 h, second polar body extrusion; 29 h pronucleus formation; Eggs isolated from oviducts; 48 h, cleavage to two cells each with a pronucleus; 72 h, 4 cell parthenogenone.

METHODS. Eggs were isolated from Graafian follicles and cultured as described in ref. 14. Egg isolation from oviducts was performed as outlined in ref. 23.

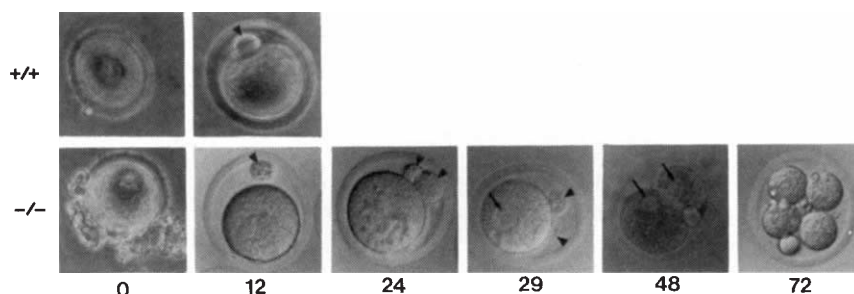


TABLE 1 Incidence of parthenogenetic activation in unfertilized eggs

Time post hCG	Genotype	Total no. of eggs			
		Class 0	Class 1	Class 2	Class 3
18 h	+/+	10	20	0	0
	-/-	0	6	33	0
32 h	+/+	0	80	0	2
	+/-	0	115	0	0
	-/-	0	0	42	129

Four-week-old mice were superovulated by PMS and hCG administration and eggs flushed from oviducts at the times indicated into M16 medium. The eggs were scored immediately into the following classes: class 0, germinal vesicle breakdown complete, no polar body; 1, eggs with a single polar body; 2, eggs with two polar bodies; 3, eggs showing cytoplasmic fragmentation.

tion of $pp39^{mos}$ we superovulated 4-week-old virgin females and scored for the presence of eggs that had failed to arrest.

Eggs isolated from mutant animals 18 h after human chorionic gonadotropin (hCG) administration predominantly had two polar bodies and no pronuclei (Table 1). Hoechst staining indicated that both polar bodies contained DNA. Eggs isolated from normal litter mates only had a single polar body. When mutant eggs with two polar bodies were maintained in culture, about 40% developed a pronucleus (Fig. 2), indicative of true parthenogenetic development. About 30% of these cleaved to two

cells, each containing a nucleus (Fig. 2), and occasionally to four cells. Development was not observed past the four-cell stage, consistent with the limited developmental potential of haploid parthenogenones. Wild-type eggs never formed second polar bodies. Mutant eggs that failed to cleave often underwent cytoplasmic fragmentation which was rarely observed in wild-type eggs. This was most noticeable in eggs isolated 32-h after the hCG injection (Table 1). We only observed parthenogenetically activated eggs in homozygous mutant animals, demonstrating that $pp39^{mos}$ is involved in maintaining metaphase II arrest. We suggest that the small numbers of normal offspring that arise from $mos^{-/-}$ mothers may be derived from eggs that were fertilized shortly after maturation, and before parthenogenetic activation renders them incapable of being fertilized.

In the LT/Sv strain of mice, 10% of oocytes undergo spontaneous parthenogenetic activation¹⁸. These mice develop teratomas containing a variety of different cell types. Importantly, we found ovarian cysts in five mutant animals but never in age-matched $mos^{+/+}$ (19 animals) or $mos^{+/-}$ (10 animals) controls. The incidence of the cysts was high (4/7) in animals older than six months but we found a small (<2 mm diameter) cyst in an animal as young as one month. The cysts were typically serous, bilateral, multilocular in older mice and in some cases (2/4) infiltrated with blood. Two types of cyst were observed. The more common (3/5) simple smaller cysts were composed of predominantly epithelial tissue (Fig. 3a) but the larger cysts (2/5), which appeared to develop at a site internal to the ovary, consisted of several tissue types typical of a teratoma. These included simple columnar epithelia, pigmented epithelia syncytial cells with large nuclei very similar in appearance to trophoblast giant cells (Fig. 3b) and folliculated tissue delineated by a basement membrane (Fig. 3d) containing eosinophilic colloid (Fig. 3c, d). This latter tissue, at least superficially, has a thyroid-like appearance but this remains to be confirmed immunohistologically. Within areas of this tissue we also observed hyperplasia of secretory epithelia forming papilliform processes and showing active resorption of colloid material (Fig. 3e, f).

These data demonstrate genetically that one function for $pp39^{mos}$ is to arrest developing mammalian eggs during meiosis before fertilization and that it is essential for mammalian cyto-static activity. Eggs lacking a functional $pp39^{mos}$ undergo spontaneous parthenogenetic activation, as defined by completion of the second meiotic division, extrusion of the second polar body and pronucleus formation without fertilization. The mutant mice also develop ovarian cysts, some of which consist of several tissue types including possible thyroid tissue. In humans, about 10% of all benign cystic teratomas of the ovary contain thyroid tissue¹⁹ and it will be of interest to investigate the status of the *c-mos* locus in these tumours and to analyse the $mos^{-/-}$ mice further to see if they provide an animal model for this type of human ovarian pathology. □

Received 20 January; accepted 12 May 1994.

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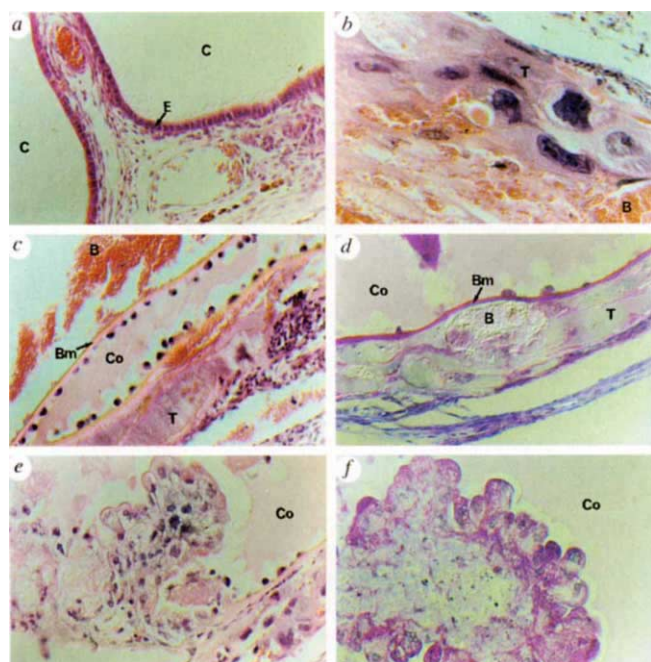


FIG. 3 Histology of ovarian cysts a, Columnar epithelium (E) lining the wall of a simple cyst (C) ($\times 200$, haematoxylin and eosin (H and E) stain); b, trophoblast-like giant cells (T) in a blood (B) filled cyst ($\times 200$, H and E stain); c, thyroid-like tissue illustrating close association with giant trophoblast-like cells ($\times 200$, H and E stain; Co, colloid); d, thyroid-like tissue stained with periodic acid-schiff (PAS) to illustrate basement membrane (Bm) layer surrounding the follicle ($\times 200$, PAS, H and E stain); e, hyperplastic secretory epithelia infiltrating colloid material ($\times 200$, H and E stain); f, secretory epithelia stained with PAS to show glycoproteins and illustrate high level of secretory activity ($\times 400$, PAS, H and E).

METHODS. Cysts were fixed in 4% phosphate buffered formalin, embedded in paraffin wax and 10- μ m sections cut and stained.

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ACKNOWLEDGEMENTS. We thank A. Surani and R. Moor for reading the manuscript and for suggestions. This work was funded by a Lloyd's Tercentenary Fellowship (W.H.C.), the CRC and The Wellcome Trust.

Parthenogenetic activation of oocytes in *c-mos*-deficient mice

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In *Xenopus* the *c-mos* proto-oncogene product (Mos) is essential for the initiation of oocyte maturation¹, for the progression from meiosis I to meiosis II^{2,3} and for the second meiotic metaphase arrest, acting as an essential component of the cytoskeletal factor CSF^{4,5}. Its function in mouse oocytes is unclear^{6–9}, however, as is the biological significance of *c-mos* mRNA expression in testes^{1,10} and several somatic tissues^{1,10,11}. We have generated *c-mos*-deficient mice by gene targeting in embryonic stem cells. These mice grew at the same rate as their wild-type counterparts and reproduction was normal in the males, but the fertility of the females was very low. The *c-mos*-deficient female mice developed ovarian teratomas at a high frequency. Oocytes from these females matured to the second meiotic metaphase both *in vivo* and *in vitro*, but were activated without fertilization. The results indicate that in mice Mos plays a role in the second meiotic metaphase arrest, but does not seem to be essential for the initiation of oocyte maturation, spermatogenesis or somatic cell cycle.

The *c-mos*-deficient mice were generated by homologous recombination in TT2 embryonic stem (ES) cells^{12,13} (for details, see Fig. 1 legend). The disruption of the *c-mos* gene in these mice was confirmed both from its genomic structure by Southern blot analysis (Fig. 1b) and by RNase protection assay of messenger RNA from the testis and ovary (Fig. 1c), where *c-mos* mRNA is most highly expressed in adult mice¹⁰.

Both *c-mos*-deficient male and female mice were apparently normal. No histological abnormality was found in brain, heart, lung, kidney, mammary gland, epididymis or placenta, in all of which low but significant levels of *c-mos* mRNA expression have been reported^{10,11}. Testes of homozygous mutant mice were also

TABLE 1 Breeding of *c-mos*-deficient females

Number of pregnancies	Number of mice +/–	–/–	Average number of offspring +/–	–/–
0	0	10	—	—
1	0	11	—	1.7
2	5	4	6.5	1.8
3	17	0	7.2	—
4	3	0	7.1	—

Twenty-five heterozygous (+/–) or homozygous (–/–) *c-mos*-deficient females were consecutively mated with fertile normal males from 2–6 months of age. Four non-pregnant (–/–) females developed massive ovarian teratomas at 4–6 months of age. These are included in zero frequency of pregnancy. Wild-type mice were not used for breeding, but the results for heterozygous mice were normal.

histologically normal (data not shown), although strong expression of *c-mos* mRNA¹⁰ and its protein¹⁴ occur in this organ. Eleven test-mated males all fathered offspring normally, with an average litter size of 6.5. Thus Mos appears to be non-essential in somatic cell growth and male germ-cell differentiation. In contrast, fertility of *c-mos*-deficient females was very low (Table 1). Ten out of 25 females examined were infertile, and even in fertile females, the frequency of pregnancy during the mating period and the number of offspring were also markedly low compared with heterozygous females. Ovarian histology of *c-mos*-deficient females showed normally differentiated germ cells and somatic cells, but significant numbers of oocytes appeared to be parthenogenetically activated (see below: Fig. 2e, f).

To determine the stage of oocyte maturation at which Mos participates, immature oocytes were collected and cultured *in vitro*¹⁵. As shown in Fig. 3a, the oocytes from *c-mos*-deficient female mice underwent germinal vesicle breakdown (GVBD) over a similar timescale to those from heterozygous and wild-type mice. These results contradict reports in which GVBD of mouse oocytes was blocked by the electrotransferred antibody against Mos⁶, although these earlier results were dependent on the antibodies used^{6,7}.

After GVBD, oocytes from both wild-type and *c-mos*-deficient female mice progressed through the first meiotic metaphase, emitted the first polar body, reached the second meiotic metaphase in 20 hours after liberation from ovarian follicles, and remained at this stage for at least 8 hours; frequency of oocytes with polar body was 77 and 74% among 236 wild-type and 289 *c-mos*-deficient oocytes, respectively. Neither chromosome decondensation nor DNA synthesis occurred throughout the maturation process (results not shown). However, the first polar body emission in *c-mos*-deficient oocytes was reproducibly delayed compared with that in wild-type oocytes (not shown), although the emission occurred asynchronously. There have been two observations relating to the role of Mos in mouse oocyte maturation. (1) *c-mos*-specific antisense oligonucleotides injected into oocytes prevent extrusion of the first polar body⁸; as this result was obtained in relatively short-term culture (<15 h), it could correspond to a delay in the first polar body emission that we have noted. (2) *c-mos*-specific antisense oligonucleotides failed to inhibit polar body emission⁹, which is consistent with our result but the oocytes injected with the nucleotides did not appear to enter the second meiotic metaphase (see below).

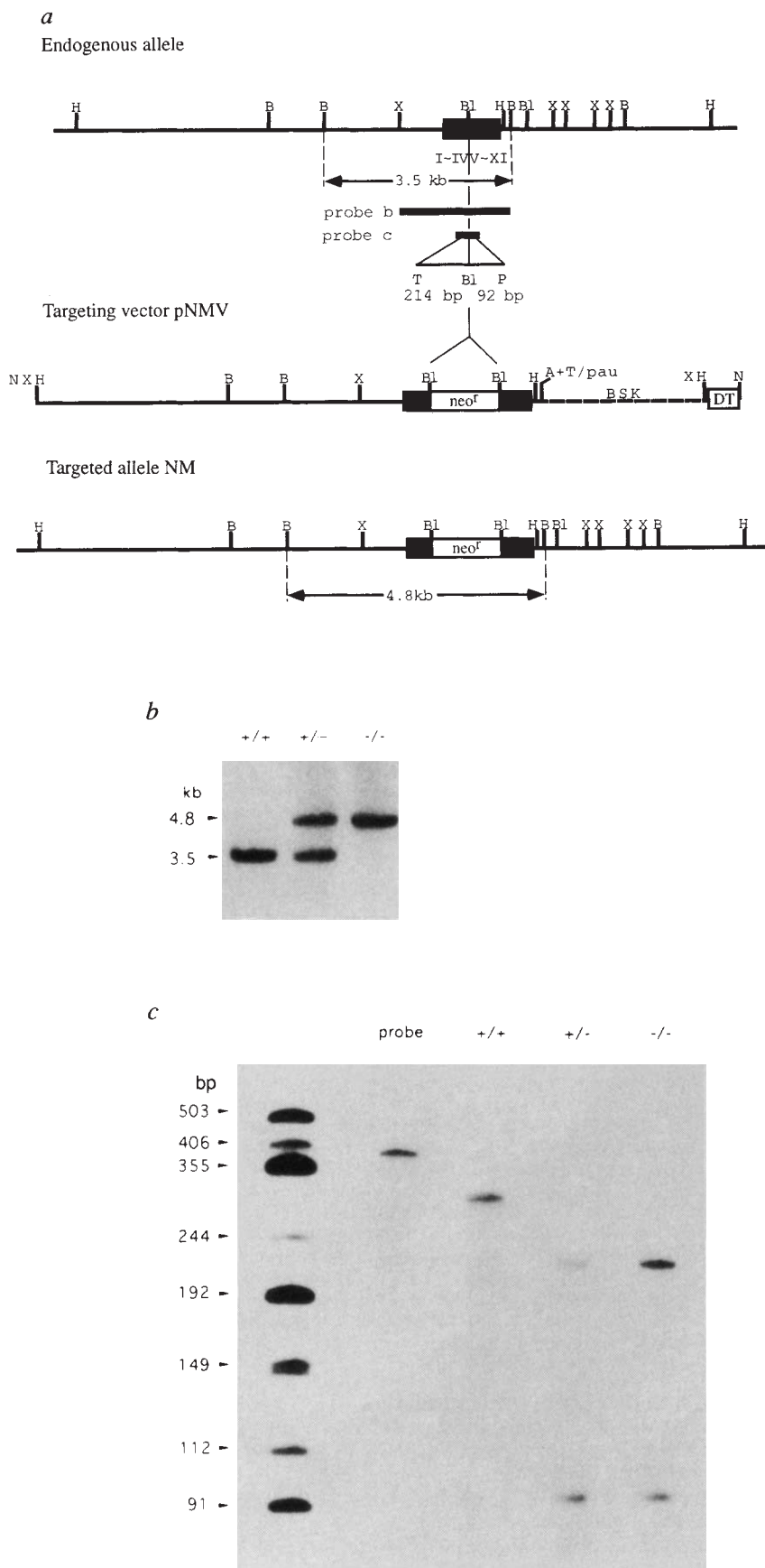
When oocytes were observed at 36–41 h after liberation from ovarian follicles, almost all wild-type oocytes remained arrested at the second meiotic metaphase, but as many as 66% of *c-mos*-deficient oocytes were released from the arrest, as evidenced by their nuclear formation (Fig. 2a, b). Most of the released oocytes had one or two nuclei, and some had divided into two or more cells. DNA synthesis was detected in nuclei of all these released oocytes, indicating their parthenogenetic activation (Fig. 2c, d). These results are apparently consistent with those from oocytes

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FIG. 1 Production of *c-mos*-deficient mice by homologous recombination in ES cells. **a**, Schematic representation of the mouse *c-mos* locus, the targeting vector pNMV and the targeted allele NM. The coding region is indicated by filled boxes. pNMV was constructed by inserting a neomycin-resistance (*neo*^r) gene with the *pgk-1* promoter in the same orientation as *c-mos* gene into the *BlnI* (*AvrII*) site in the kinase subdomain V of Mos²⁰. The N-terminal truncated product created by the disruption should contain kinase subdomains I–IV, but this product should have no kinase activity²⁰. The C terminus should be expressed as a fusion RNA with the *neo*^r gene directed by *pgk-1* gene promoter, but stop codons exist in all three frames at the end of the *neo*^r gene. Also, even if the truncated product (kinase subdomains V–XI) were translated, it would have no kinase activity²⁰. Several restriction enzyme sites, probe b (*XbaI*–*HindIII* fragment) used for Southern blot analysis in panel **b** and probe c used for the RNase protection assay in panel **c**, are shown with the predicted fragment sizes. B, *BglII*; Bl, *BlnI*; H, *HindIII*; N, *NotI*; X, *XbaI*; T, *EcoT221*; P, *PstI*. **b**, Examples of genomic Southern blot analysis on wild type (+/+), heterozygous (+/–) and homozygous (–/–) mutant mice originated from clone w8-3. Genomic DNAs were isolated from tails of mice and digested by *BglII*. **c**, RNase protection assay of *c-mos* RNA expression in testes of wild-type, heterozygous and homozygous mutant mice originating from clone w8-3. The 306-base-pair (bp) band represents a normal *c-mos* transcript, the 214-bp band corresponding to a T–Bl region represents a 5' transcript truncated by insertion of the *neo*^r gene, and the 92-bp band probably represents a transcript in fusion with the *neo*^r gene directed by the *pgk-1* promoter¹². This promoter has a strong transcriptional activity in testes. RNase protection assay was also done on ovarian RNAs with the same results, except that the 92-bp band was absent (not shown); the *pgk-1* gene promoter does not have significant activity in the ovary. Sizes of marker DNAs from ³²P-labelled *HpaII* digests of pUC18 are shown.

METHODS. A targeting vector pLMV which had a *lacZ*–*neo*^r cassette instead of the *neo*^r cassette was also constructed; the ES cells used were TT2 cells derived from an F₁ embryo between C57BL/6 and CBA mice¹³. Details of the isolation of homologous recombinants, including negative selection with A + T/pau–BSK–DT, and primers for PCR analysis are described elsewhere¹². The homologous nature of the recombination was confirmed by Southern blot analysis using several restriction enzymes and several probes located either inside or outside the targeting vectors. Eleven of the homologous recombinants were screened for multipotency by injecting the cells into 40 ICR 8-cell embryos, yielding mice whose coat colour was TT2-derived agouti¹³ for three clones with pLMV (w72-1, w74-1, w75-4) and two with pNMV (w8-3, w15-3). Mice derived from the three clones (w8-3, w72-1, w74-1) were used to produce heterozygous F₁ mice by mating with C57BL/6 females. Although the targeting vector pLMV was designed to determine the sites of endogenous Mos expression by β -galactosidase activity, no activity was detected in any tissues (reasons unknown). As no difference was found in results of the subsequent analyses among w8-3, w72-1 and w74-1 strains of *c-mos*-deficient mice, no specification was made about which strains of mice were used in each experiment. The probe for the RNase protection assay was prepared by inserting the *EcoT221*–*PstI* DNA fragment into *HindIII*–*PstI* sites of Bluescript (SK⁺) and then synthesizing antisense RNA using ³²P-UTP and phage T3 RNA polymerase after digestion by *XhoI*. 3 μ g total RNAs from testes or ovary was hybridized with the probe, digested with RNase T1, resolved by 5% acrylamide gel electrophoresis (50% (w/v) urea) and autoradiographed.



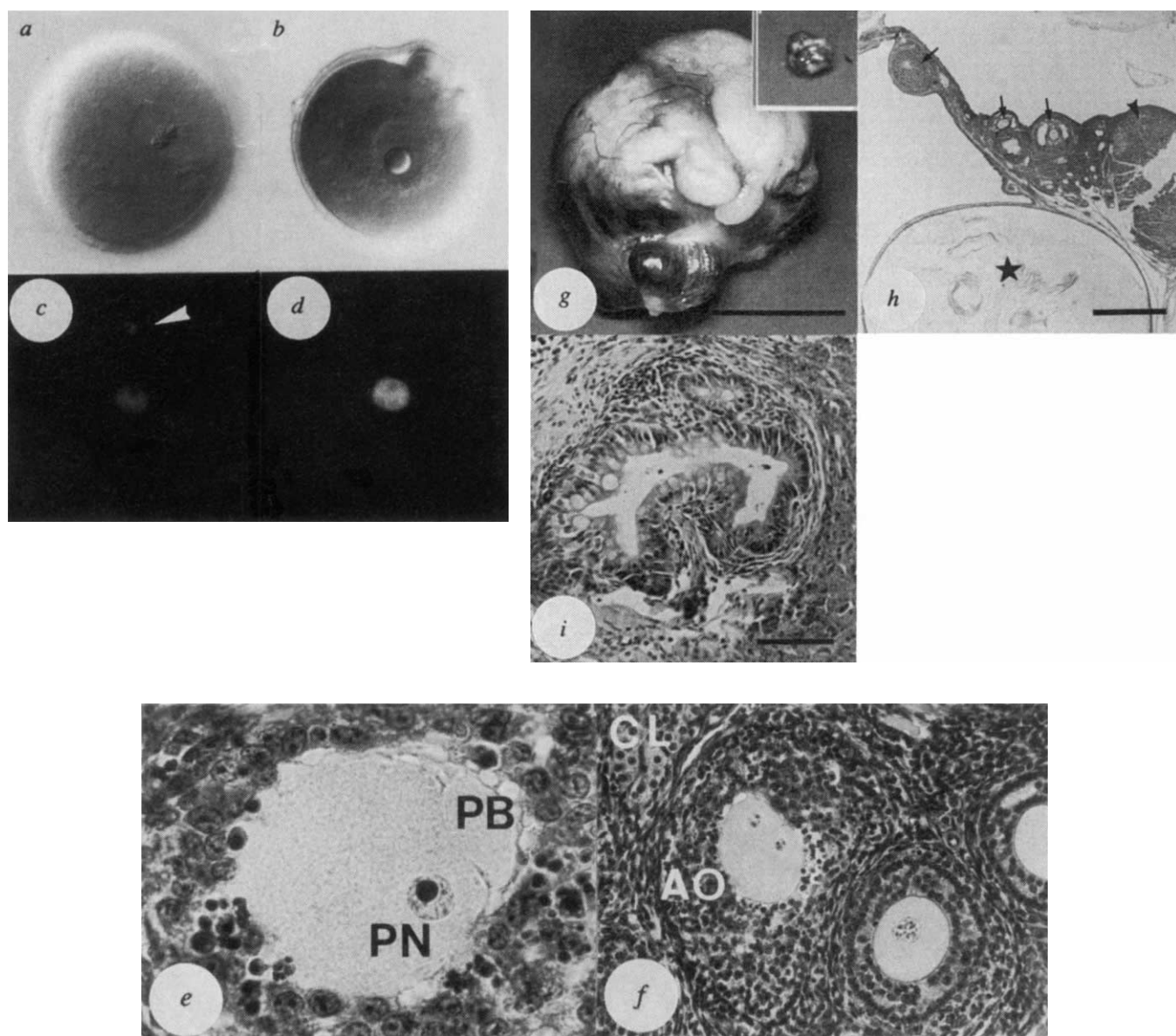


FIG. 2 Oocytes, ovaries and teratomas in *c-mos*-deficient female mice. *a*, Wild-type oocyte remaining in the second metaphase; magnification, $\times 325$. *b*, Activated *c-mos*-deficient oocyte having a nucleus with a large nucleolus, $\times 325$. *c*, *d*, DNA synthesis in activated *c-mos*-deficient oocyte at 40 h after liberation; $\times 300$. *c* and *d*, Same field: *c*, DNA staining with 4,6-diamidino-2-phenylindole (DAPI). Arrowhead indicates a polar body; *d*, DNA synthesis in pronucleus detected with anti-BrUdR antibody. *e* and *f*, Activation of ovarian oocytes in *c-mos*-deficient mice. *e*, Activated intrafollicular oocyte. PB, polar body; PN, pronucleus; $\times 580$. *f*, Ovarian architecture in *c-mos*-deficient mice. CL, corpus luteum; AO, activated oocyte; $\times 174$. *g*, Macroscopic view of a large ovarian tumour with numerous vascularizations. Inset shows the normal size of an ovary at the same magnification. *h*, Example of histological sections of moder-

ately enlarged ovaries with columnar epithelial cysts containing keratin (star); arrows, follicles; arrowhead, corpus luteum. Note the development of the teratoma within the ovary. *i*, Histological view of enlarged tumour showing organization, glandular epithelium and melanin deposit. Scale bars in *g-i* are 1 cm, 300 μ m and 30 μ m, respectively.

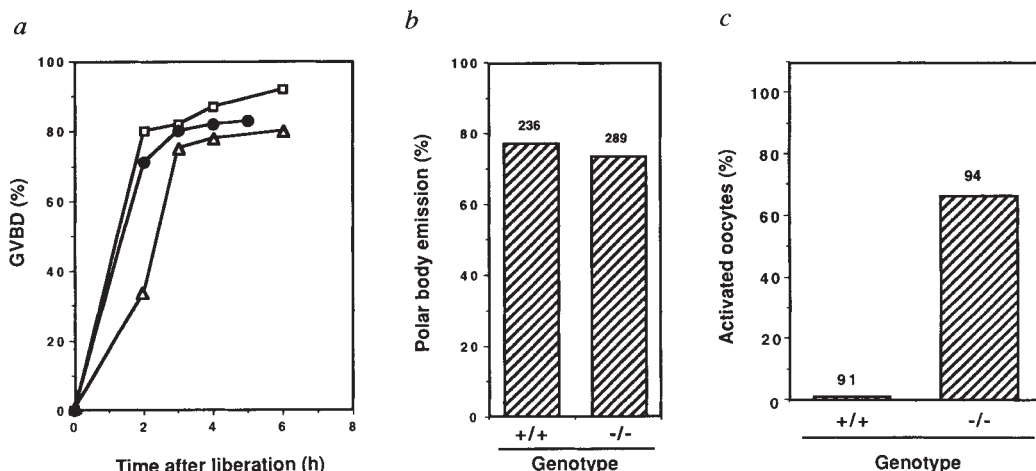
METHODS. *a*, *b*, Oocytes were cultured *in vitro* for 36–41 h after liberation, then fixed and stained as described²¹. *c*, *d*, Immature oocytes were cultured in the presence of 0.4 mM BrUdR and 0.1 mM uridine for 40 h. DNA staining with DAPI and detection of incorporated BrUdR with anti-BrUdR antibody were performed on 19 oocytes as described²⁴. *e*, *f*, *h*, *i*, Ovaries were fixed with 10% formalin, embedded in paraffin and serially sectioned. Specimens were stained with haematoxylin and eosin.

injected with antisense oligonucleotides⁹, although nuclei were reformed within 2–3 h after emission of the first polar body in those oocytes. In addition, when *in vivo* ovulated oocytes were collected 17–21 h after human chorionic gonadotropin (hCG) injection, about 63% of them were activated, as determined by their nuclear formation (Fig. 3*b*). In the ovary, many oocytes were not only activated with nuclear formation (Fig. 2*e*), but also underwent several divisions. These results indicate that the *c-mos*-deficient oocytes are readily activated parthenogenetically before or just after ovulation.

We also investigated the fertility of *c-mos*-deficient oocytes *in vitro*. Thirty-seven per cent of the ovulated oocytes had not been activated (Fig. 3*b*), as evidenced by the presence of metaphase chromosomes and spindles. Successful fertilization occurred, however, in only 11% of ovulated oocytes, as shown by the presence of sperm heads in the cytoplasm (Fig. 3*c*). Thus even among oocytes with metaphase chromosomes and spindles, most had lost the capacity for penetration by sperm. This is unlikely to be due to a defect in the zona pellucida: sperm were found in the perivitelline space of the unfertilized oocytes; and a simi-

FIG. 3 *In vitro* maturation and fertility of *c-mos*-deficient oocytes. **a**, Time course of GVBD of oocytes after liberation from ovarian follicles. Squares, triangles and circles represent oocytes from wild-type, heterozygous mutant and homozygous mutant mice, respectively. **b**, Spontaneous activation of ovulated oocytes. **c**, Fertility of *c-mos*-deficient oocytes. +/+, Wild type; -/-, homozygous mutant; 'zona-free', oocytes freed of zona pellucida before insemination. Number at the top of each column indicates the total number of oocytes examined.

METHODS. **a**, Immature oocytes were liberated from ovarian follicles and cultured as described¹⁵. GVBD was examined under differential interference optics. More than 100 oocytes were examined at each time point for wild type and homozygous mutants, and 41 oocytes were examined for heterozygous mutants. **b**, 8–12-week-old *c-mos*-deficient mice were primed with hCG (8 i.u.) 48 h after treatment with pregnant mare serum gonadotropin (8 i.u.). Ovulated oocytes were collected from oviducts 17–21 h after hCG injection, fixed and stained²¹.



Activation of oocytes was determined by their nuclear formation. **c**, Ovulated oocytes were collected 15 h after hCG injection. The zona pellucida was removed by treatment with acidic Tyrode solution²². Zona-free and intact oocytes were inseminated with capacitated sperm of B6C3F1 mice *in vitro*²³. Fertilization was determined by the cytoplasmic location of enlarged or partially decondensed sperm heads under a differential interference microscope.

larly low fertility was found in *c-mos*-deficient oocytes free of zona pellucida (Fig. 3c). Changes in the plasma membrane might occur at an earlier step of parthenogenetic activation; normally the plasma membrane block occurs ~40 min after fertilization in zona pellucida-free mouse oocytes¹⁶, and the zona reaction 5–10 h after fertilization¹⁷.

The development of ovarian teratoma is closely related to the parthenogenetic activation of oocytes¹⁸. Indeed, the *c-mos*-deficient female mice frequently developed ovarian teratomas (Fig. 2g–i): of 25 female mice (Table 1), massive ovarian tumours had developed in seven at 4–9 months of age, and were found in three more upon anatomical investigation.

In *Xenopus*, Mos functions at several steps in oocyte maturation^{1–3,5}, but in mice it acts almost exclusively in the second meiotic metaphase arrest, being an essential component of CSF⁵. The role of Mos is probably to prevent parthenogenetic activation, as recently demonstrated in *Xenopus*¹⁹. Our *c-mos*-deficient mice could be useful for studying the mechanisms of oogenesis and teratogenesis in mammals and infertility in women. □

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ACKNOWLEDGEMENTS. N.H. and N.W. contributed equally to this work. We thank T. Yagi, S. Nada and N. Kohmura for plasmids; Y. Shigetani and K. Iwasaki for technical assistance; and A. Hunter for critically reading the manuscript. This work was supported in part by grants-in-aid from the Science and Technology Agency and the Ministry of Education, Science and Culture of Japan.

PDGF- and insulin-dependent pp70^{S6k} activation mediated by phosphatidylinositol-3-OH kinase

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PLATELET-DERIVED growth factor receptor (PDGF-R) phosphorylation at tyrosines 740/751 and insulin receptor phosphorylation of insulin receptor substrate-1 effects the recruitment and activation of phosphatidylinositol-3-OH kinase (PI(3)K)^{1–5}. Changes in PI(3)K activity correlate with cell growth but its downstream signal transducers are unknown^{4,5}. Activation of the 70/85K S6 kinases (pp70^{S6k}) by serine phosphorylation^{6,7} results in 40S ribosomal protein S6 phosphorylation and is important for G1 cell-cycle transition in a variety of cells^{8–11}. Although receptor tyrosine kinases activate the microtubule-associated protein kinase cascade through SH2-/SH3-adaptor proteins, Sos and c-Ras¹², it is unclear

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Received 2 February; accepted 8 June 1994.

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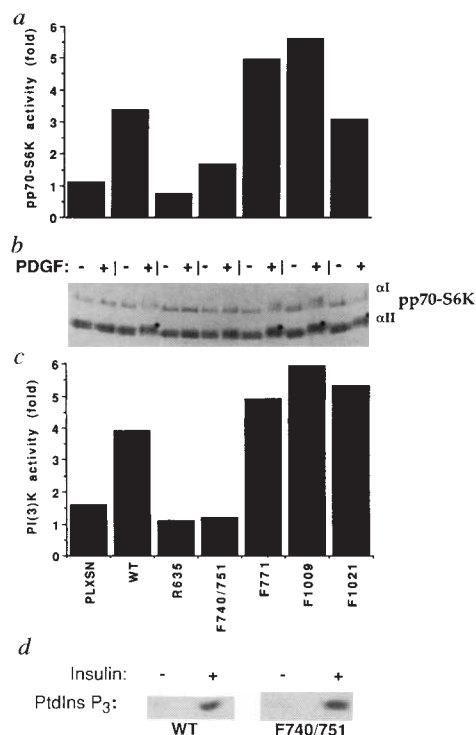


FIG. 1 Tyrosine phosphorylation of PDGF-R binding sites for PI(3)K is coupled to activation of pp70^{S6K}. **a**, PDGF-dependent pp70^{S6K} activation in HepG2 cells expressing the pLXSN vector, or WT, R635, F740/751, F771, F1009, or F1021 PDGF-Rs¹³. Results are presented as fold increase of activity over untreated cells. PDGF-dependent RSK activation was consistently observed in these cells (not shown), indicating that pp70^{S6K} inhibition is not due to disruptions of broad signalling pathways. **b**, Phosphorylation of pp70^{S6K} (α II form) and pp85^{S6K} (α I form, see also Figs 3 and 4) as examined by immunoblot analysis. pp70^{S6K} isoforms result from alternative translational start sites²⁹ and activation correlates with the slowest migrating species on SDS-polyacrylamide gels (indicated with dots). **c**, PI(3)K activity associated with the PDGF-R mutants. **d**, Insulin-dependent PI(3)K activation in HepG2 cells expressing wild-type and F740/751 receptors.

METHODS. **a**, **b**, Quiescent HepG2 cells expressing various PDGF-Rs were stimulated with PDGF-BB (40 ng ml⁻¹) (45 min), and pp70^{S6K} activity (**a**) and extent of phosphorylation (**b**) were measured⁸. pp70^{S6K}-specific activity for unstimulated cells expressing pLXSN vector, or WT, R635, F740/751, F771, F1009 or F1021 PDGF-Rs were 37.4, 40.8, 18.6, 53.2, 36.6, 35.7 and 38.8 pmol min⁻¹g⁻¹ (total cell lysate protein), respectively. Standard deviations ($n=3$) were 0.17-, 0.45-, 0.18-, 0.16-, 1.47-, 0.59- and 0.17-fold, respectively. The PDGF-R β -mutants have phenylalanine replacing tyrosine at positions 740 and 751, 771, 1,009 or 1,021¹³. **c**, PI(3)K activity associated with PDGF-R mutants. PI(3)K activity in PDGF-R or anti-phosphotyrosine immunoprecipitates (not shown) was measured³⁰ from cells stimulated with PDGF (7.5 min) in parallel with the pp70^{S6K} experiments. **d**, Quiescent cells were stimulated (7.5 min) with insulin (10⁻⁷ M), and PI(3)K activity measured in anti-phosphotyrosine immunoprecipitates³⁰.

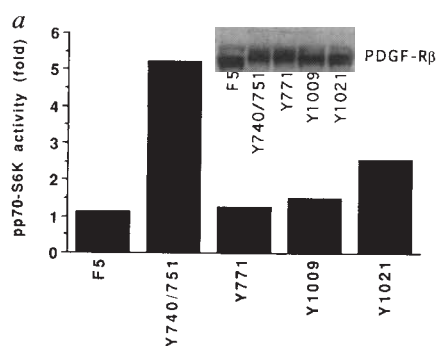
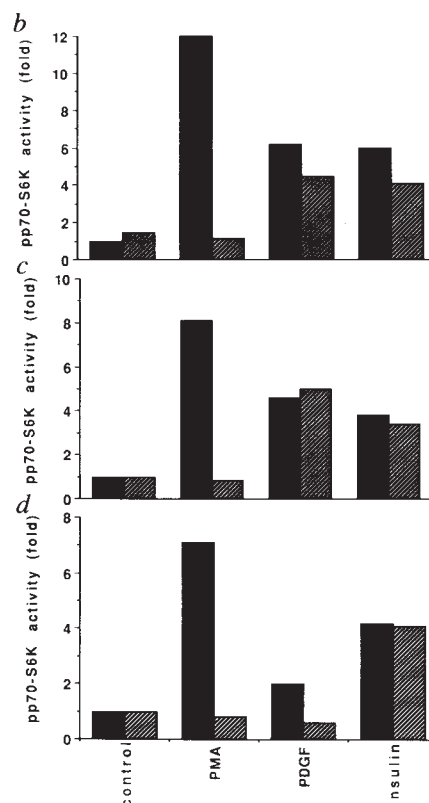


FIG. 2 **a**, Tyrosine phosphorylation of PDGF-R binding sites for PI(3)K or PLC- γ are sufficient for pp70^{S6K} activation. PDGF-stimulated pp70^{S6K} activity in cells expressing F5, Y740/751, Y771, Y1009, or Y1021 PDGF-Rs¹³ was measured. The results are presented as fold activation over untreated cells. Insert, Expression levels of the different Y-mutant PDGF-Rs were determined by immunoblot analyses. **b–d**, The role of cPKC in PLC- γ - or PI(3)K-mediated activation of pp70^{S6K}. PMA-, PDGF-, insulin-stimulated pp70^{S6K} activities were measured in the HepG2 cells expressing WT (**b**), Y740/751 (**c**), or Y1021 (**d**) PDGF-R β . Hatched bars, cells pretreated with PMA to effect cPKC downregulation and subsequently incubated with indicated mitogen. Black bars, mitogen-dependent activation without cPKC downregulation.

METHODS. **a**, Quiescent HepG2 cells expressing various PDGF-R mutants were stimulated with PDGF-BB (40 ng ml⁻¹, 45 min) and pp70^{S6K} activities were measured⁸. pp70^{S6K}-specific activity from unstimulated cells expressing F5, Y740/751, Y771, Y1009, or Y1021 PDGF-Rs was 13.9, 7.3, 5.2, 7.7, and 8.2 pmol min⁻¹g⁻¹ (total cell lysate protein), respectively. Standard deviations ($n=3$) for each cell line were 0.14-, 1.94-, 0.04-, 0.09-, and 0.06-fold, respectively. F5 is the PDGF-R β with Tyr 740/751, 771, 1009, and 1,021 mutated to phenylalanine. Y740/751, Y771, Y1009 and Y1021 are variations of F5 which have tyrosine replacing phenylalanine at positions 740/751, 771, 1,009 or 1,021, respectively¹³. **b–d**, HepG2 cells expressing PDGF-Rs were grown to confluence, then serum-starved for 2 days. PKC isozymes were depleted



by PMA (100 ng ml⁻¹) preincubation for the final 20 h of starvation (hatched bars). Control cells were treated with the vehicle DMSO (0.1%) (black bar). Cells were stimulated with PMA (100 ng ml⁻¹), PDGF (40 ng ml⁻¹) or insulin (10⁻⁷ M) for 45 min and pp70^{S6K} activities measured⁸. These results are representative of three independent experiments.

how tyrosine kinases are coupled to the pp70^{S6k} phosphorylation cascade. Here we report that PI(3)K mediates PDGF or insulin receptor signalling to pp70^{S6k}. PI(3)K-mediated activation of pp70^{S6k} is independent of conventional protein kinase C isoforms. Additionally, rapamycin blocks pp70^{S6k} activation by all mitogens⁸⁻¹⁰, without inhibiting PI(3)K, and acts downstream in this signalling system.

To examine PDGF-dependent pp70^{S6k} activation, we used HepG2 cells expressing either (1) mutant PDGF-Rs unable to associate with the GTPase-activating protein acting on a *ras* protein (rasGAP), SHPTP2 (SH2-containing protein tyrosine phosphatase 2), phospholipase C γ (PLC- γ) or PI(3)K; (2) PDGF-R 'add-back' mutants that associate with only one of the above; or (3) a kinase-inactive PDGF-R mutant^{1,13}. PDGF activated pp70^{S6k} in cells expressing wild-type (WT) human PDGF-R β but not in cells expressing vector alone (pLXSN) or the kinase-inactive (R635) receptor (Fig. 1a). PDGF-dependent pp70/85^{S6k} phosphorylation (Fig. 1b) and activation (Fig. 1a) was noticeably reduced in cells expressing the PDGF-R mutant unable to bind PI(3)K (F740/751). As expected, these cells exhibited little PDGF-mediated activation of PI(3)K activity (Fig. 1c). However, insulin, which activates PI(3)K through the 180K insulin receptor substrate-1 (IRS-1)⁵, activated PI(3)K, demonstrating intact signal transduction in F740/751 cells (Fig. 1d).

The F740/751 mutant, which associates with PLC- γ , can moderately activate pp70^{S6k}. Stimulation of the F1021 mutant, which fails to associate with PLC- γ , does not fully activate

pp70^{S6k} as seen in the F771 or F1009 mutants, even though PI(3)K activities are equivalent (compare Fig. 1a and c). Thus, PLC- γ also contributes to PDGF-mediated activation of pp70^{S6k}, consistent with the observation that phorbol esters stimulate pp70^{S6k} activity in cultured cells¹⁴.

To determine if PI(3)K or PLC- γ activation is sufficient for pp70^{S6k} activation, PDGF-R add-back mutants¹³ were used. Activation of PI(3)K through the PDGF-R (Y740/751) resulted in a substantial pp70^{S6k} activation (5–7-fold, Fig. 2a). A smaller (2–3-fold), yet reproducible, stimulation was observed with the PLC- γ (Y1021) add-back. No apparent activation was mediated by the rasGAP (Y771) or SHPTP2 (Y1009) add-backs. Notably, only PI(3)K or PLC- γ add-back mutants stimulate growth¹⁴.

We next asked if PDGF-R (Y740/751) phosphorylation activates pp70^{S6k} through a conventional protein kinase C (cPKC)-dependent or -independent mechanism. Pp70^{S6k} activation by the PI(3)K, but not the PLC- γ add-back mutant, was insensitive to cPKC downregulation (Fig. 2c, d). Insulin-dependent pp70^{S6k} activation is also largely cPKC-independent in cells expressing the WT and Y740/751 receptor mutants (Fig. 2b, c). Insulin activates PI(3)K¹⁵ but not PLC- γ ¹⁶, supporting a cPKC-independent link between PI(3)K and pp70^{S6k}.

To support our genetic evidence coupling PI(3)K to the pp70^{S6k} signalling system, we used wortmannin, a potent PI(3)K inhibitor^{17,19}. The 50% inhibitory concentration (IC₅₀) for inhibition of PDGF-stimulated pp70^{S6k} activity (Fig. 3a) and phosphorylation (Fig. 3b) was less than 10 nM *in vivo*, similar to that for PI(3)K *in vitro* (Fig. 3a) and for formation of phosphati-

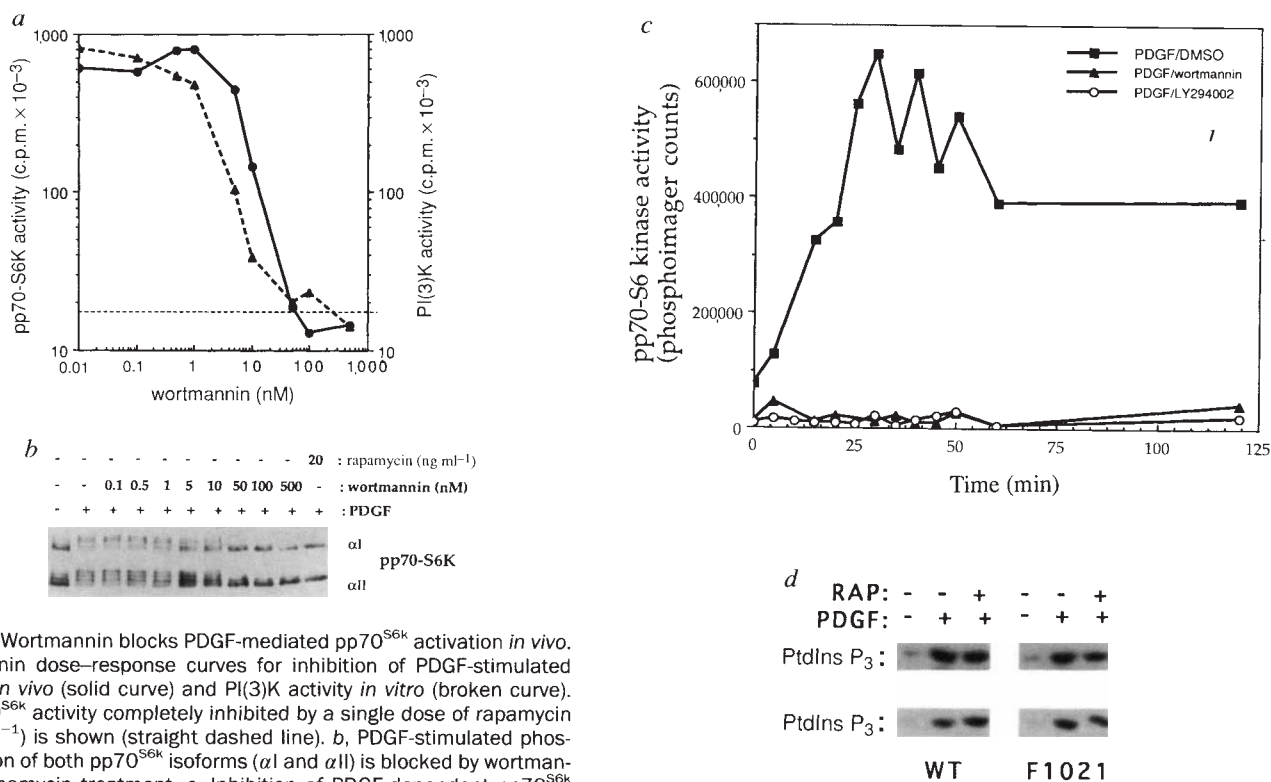


FIG. 3 a, Wortmannin blocks PDGF-mediated pp70^{S6k} activation *in vivo*. Wortmannin dose-response curves for inhibition of PDGF-stimulated pp70^{S6k} *in vivo* (solid curve) and PI(3)K activity *in vitro* (broken curve). The pp70^{S6k} activity completely inhibited by a single dose of rapamycin (20 ng ml⁻¹) is shown (straight dashed line). b, PDGF-stimulated phosphorylation of both pp70^{S6k} isoforms (α 1 and α 2) is blocked by wortmannin or rapamycin treatment. c, Inhibition of PDGF-dependent pp70^{S6k} activation throughout early G1 wortmannin or LY294002. d, Rapamycin does not inhibit agonist-stimulated PI(3)K activity. PDGF-stimulated PI(3)K activities associated with either anti-phosphotyrosine (top panel) or anti-PDGF-R β (bottom panel) immunoprecipitates were measured from WT and F1021 HepG2 cells pretreated with (+) or without (-) rapamycin.

METHODS. a, Quiescent HepG2 cells expressing the Y740/751 PDGF-R mutant were pretreated with vehicle (DMSO) or various concentrations of wortmannin for 5 min, and then stimulated with PDGF-BB (40 ng ml⁻¹, 45 min) and pp70^{S6k} activity measured⁸. PI(3)K activity associated with PDGF-R was measured in a parallel experiment with cells treated with PDGF for 5 min. PI(3)K activity was measured as

described³⁰ except with reaction buffer containing the vehicle DMSO (0.1%) or various concentrations of wortmannin. b, The phosphorylation and amount of pp70^{S6k} in a was determined by immunoblot analysis⁸. c, Quiescent HepG2 cells expressing the wild-type PDGF-R were incubated with the vehicle DMSO (0.1%), wortmannin (100 nM) or LY294002 (50 μ M) for 20 min, and stimulated with PDGF-BB (40 ng ml⁻¹) for various times. Similar results were observed with the PDGF-R Y740/751 add-back supporting the notion that this activation period is largely dependent on PI(3)K. d, Quiescent cells were stimulated with PDGF (+, 40 ng ml⁻¹, 7.5 min) with (-) or without (+) rapamycin (RAP, 20 ng ml⁻¹) pretreatment (20 min) and PI(3)K activities measured³⁰.

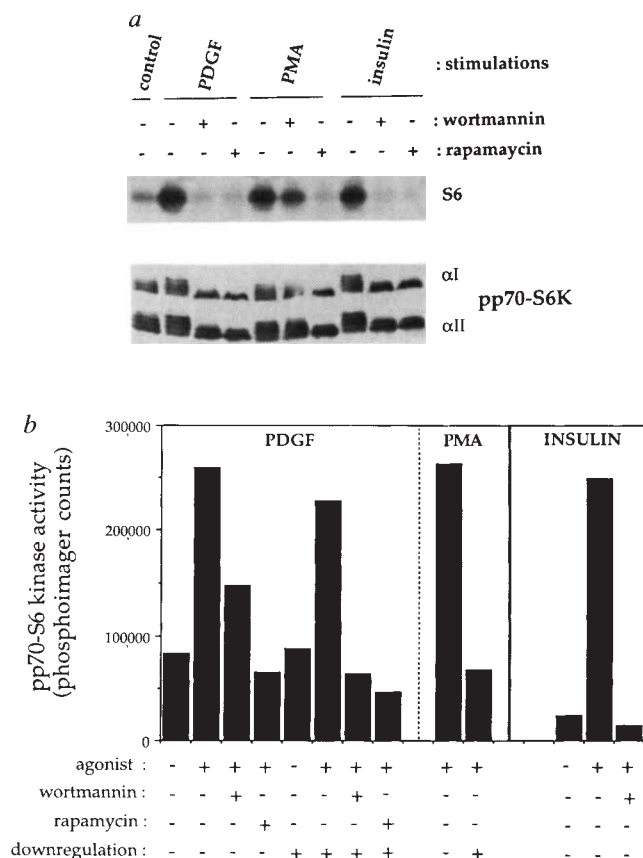


FIG. 4 **a**, Wortmannin inhibits PDGF- and insulin-dependent, but not PMA-stimulated, activation of pp70^{S6K} in HepG2 cells. Top panel, pp70^{S6K} activity as measured in the immune complex⁸. Bottom panel, pp70^{S6K} immunoblot analysis for the cell lysates. **b**, PI(3)K activation of pp70^{S6K} is not limited to HepG2 cells. Wortmannin completely inhibits the cPKC-independent activation of pp70^{S6K} by PDGF in Swiss 3T3 fibroblast cells (see PDGF and PMA data in the figure) and completely inhibits insulin activation of pp70^{S6K} in myeloid progenitor cells (see insulin data in figure).

METHODS. **a**, Quiescent HepG2 cells expressing the Y740/751 PDGF-R mutant were treated with wortmannin (50 ng ml⁻¹, lanes 3, 6 and 9), rapamycin (20 ng ml⁻¹, lanes 4, 7 and 10), or with the vehicle DMSO (lanes 2, 5 and 8) for 5 min. They were then stimulated with PDGF (40 ng ml⁻¹, lanes 2, 3 and 4), PMA (100 ng ml⁻¹, lanes 5, 6 and 7), or insulin (10⁻⁷ M, lanes 8, 9 and 10) for 45 min. pp70^{S6K} activity and immunoblot analysis was completely as described⁸. **b**, Swiss 3T3 fibroblasts (PDGF and PMA bars in figure) were made quiescent by 48 h serum deprivation. cPKC downregulation was successfully completed by administering PMA (100 ng ml⁻¹) for the last 20 h of starvation (see PMA control). Cells were then treated with the vehicle DMSO (0.1%), wortmannin (100 nM), or rapamycin (20 ng ml⁻¹) for 5 min prior to PDGF (40 ng ml⁻¹) stimulation for 30 min (PDGF bars). 32D myeloid precursor cells ectopically expressing the insulin receptor and insulin receptor substrate-1 (a gift from M. White and J. Pierce) were treated with vehicle (0.1% DMSO) or wortmannin (100 ng ml⁻¹) for 10 min and then stimulated with insulin (10⁻⁷ M) for 30 min (insulin bars). Cells were lysed and assayed for pp70^{S6K} activity as described⁸.

diinositol-3,4,5-triphosphate (PtdIns (3,4,5)P₃) in HepG2 cells (1–10 nM; L. Cantley, personal communication) and other cell types¹⁹. Wortmannin did not inhibit pp70^{S6K} *in vitro* at concentrations up to 1 μ M (not shown).

Because wortmannin might inhibit an unknown pp70^{S6K}-activating kinase, we also used a structurally unrelated PI(3)K-specific inhibitor. LY294002 inhibits PI(3)K at concentrations (10–100 μ M) that like wortmannin, does not inhibit pp70^{S6K} or other protein kinases^{20,21}. pp70^{S6K} has been reported to exhibit biphasic activation kinetics²², during which PI(3)K may be only partly involved. However, both inhibitors block pp70^{S6K} activation by PDGF (Fig. 3c) and insulin²¹ throughout early G1. The basal activity was also inhibited (see also Figs 3 and 4), possibly due to high basal PI(3)K activity in HepG2 cells (L. Cantley, personal communication).

Pp70^{S6K} is activated by cPKC-dependent and -independent mechanisms¹⁴ (Figs 2 and 4), and rapamycin blocks both pathways⁸. Rapamycin, however, does not inhibit PI(3)K *in vivo* or *in vitro* (Fig. 3d and not shown). Wortmannin, although inhibiting PDGF (Y740/751)- and insulin-mediated activation of pp70^{S6K} in HepG2 cells, did not similarly block phorbol ester (cPKC)-mediated activation (Fig. 4a). The partial inhibition observed is probably due to the basal PI(3)K activity discussed above and its contribution to total pp70^{S6K} activity. Thus, PI(3)K modulates cPKC-independent signalling to pp70^{S6K}. These observations are not specific to HepG2 cells because wortmannin inhibits cPKC-independent activation of pp70^{S6K} in PDGF-stimulated Swiss 3T3 fibroblasts and in insulin-stimulated myeloid cells ectopically expressing the insulin receptor and IRS-1 (Fig. 4b). Our results are not limited to receptor tyrosine kinases as interleukin-2 (IL-2)-dependent activation of pp70^{S6K} is also antagonized by wortmannin (not shown).

This study identifies the first, to our knowledge, downstream molecular target of PI(3)K, namely pp70^{S6K}. It is possible that PDGF-dependent signalling to pp70^{S6K} is through Nck binding

to Y751²³, however, PDGF-dependent pp70^{S6K} activation in wild-type versus Nck-overexpressing NIH3T3 cells are equivalent, and cells morphologically transformed by Nck do not exhibit constitutively activated pp70^{S6K} (not shown). Unknown factors may participate in this pathway, such as other signalling proteins that may bind to the PI(3)K regulatory or catalytic subunits. Experiments aimed at elucidating how this signal is mediated are underway. One potential intermediate is the phorbol ester-insensitive ζ isoform of PKC which is activated by PtdIns (3,4,5) P₃, one of the products of PI(3)K activation, *in vitro*²⁴.

Besides potentially regulating protein translation through S6 phosphorylation¹⁴, pp70^{S6K} may couple PI(3)K to the regulation of gene expression. For example, glycogen synthase kinase 3 is inhibited by pp70^{S6K} *in vitro*²⁵, which may result in specific dephosphorylation and activation of c-Jun *in vivo*²⁶. Wortmannin also antagonizes IgE-mediated histamine release¹⁸ and insulin-dependent GLUT4 translocation^{17,21}. However, rapamycin antagonizes neither process^{27,28}, suggesting that PI(3)K-regulated signalling bifurcates upstream of pp70^{S6K}, with the branched pathway potentially regulating protein sorting/secretion. Clearly, further characterization of this signalling system will improve our understanding of the regulation of cell proliferation and cellular metabolism. □

Received 27 January; accepted 26 May 1994.

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ACKNOWLEDGEMENTS. We thank M. F. Birnbaum, L. C. Cantley, M. M. Chou, B. Drucker, H. Hanafusa, J. Pierce, T. Roberts, S. N. Seghal, C. H. Vlahos, M. White, M. Whitman and members of the Blenis laboratory for reagents and discussions. This work was supported by the NIH (J.B. and A.K.), Harvard Medical School Funds for Discovery (J.B.) and the Lucille P. Markey Charitable Trust (J.B.). J.C. is an Albert J. Ryan fellow and supported by a Lucille P. Markey predoctoral fellowship. K.P.L. is a Howard Hughes Medical Institute Medical Student Research Training Fellow.

Phosphorylation of RNA polymerase II C-terminal domain and transcriptional elongation

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THE carboxy-terminal domain (CTD) of the large subunit of RNA polymerase II is essential *in vivo*^{1–4}, and is found in either an unphosphorylated (IIa) or hyperphosphorylated (IIo) form^{5,6}. The *Drosophila* uninduced *hsp70* and *hsp26* genes, and the constitutively expressed β -1 tubulin and *Gapdh-2* genes, contain an RNA polymerase II complex which pauses after synthesizing a short transcript^{7–10}. We report here that, using an *in vivo* ultraviolet crosslinking technique¹¹ and antibodies directed against the IIa and IIo forms of the CTD¹², these paused polymerases have an unphosphorylated CTD. For genes containing a 5' paused polymerase, passage of the paused RNA polymerase into an elongationally competent mode *in vivo* coincides with phosphorylation of the CTD. Also, the level of phosphorylation of the CTD of elongating polymerases is shown not to be related to the level of transcription, but is promoter specific.

We have determined the phosphorylation state of the CTD of both paused and elongating polymerases, and provide here what we believe to be the first high-resolution view of the step in transcription when phosphorylation of the CTD occurs *in vivo*. An *in vivo* protein–DNA crosslinking assay¹¹ was used where the different forms of RNA polymerase crosslinked to DNA were immunoprecipitated with antibodies directed against the unphosphorylated (IIa antibody) and the phosphorylated (IIo antibody) forms of the *Drosophila* CTD¹². The IIo antibody shows strong specificity for the highly phosphorylated form of the CTD, whereas the IIa antibody is highly selective for the

unphosphorylated CTD; both antibodies, however, can recognize a partially phosphorylated CTD (see below and ref. 12).

In uninduced cells, the IIa, but not IIo, antibody precipitated complexes containing DNA corresponding to the 5' half of the *hsp70* gene (Fig. 1a; and Fig. 1b, lanes 5 and 6). Neither antibody precipitated DNA from the 3' half of the gene. The RNA polymerase II associated with the 5' half of the uninduced *hsp70* gene has been mapped to the region 20 to 40 bases downstream of the transcription start site^{7,9}. The failure of the IIo antibody to precipitate complexes is not due to the absence of DNA in the IIo precipitated sample, because strong hybridization in this lane is seen when the filter is reprobed to detect the 5' 2.4 kilobases (kb) of the constitutively expressed *hsp83* gene. The paused polymerase at the 5' end of the *hsp70* gene must contain a CTD which is either unphosphorylated or phosphorylated at a level that is too low to be detected by the IIo antibody.

In heat-shock-induced cells, transcription of the *hsp70* gene increases about 300-fold¹³. Both the IIa and IIo antibodies immunoprecipitate complexes containing DNA corresponding to the *hsp70* gene (Fig. 1b, lanes 11 and 12), indicating that the level of phosphorylation of the CTD increases after heat shock. However, some of these elongating polymerases could have a CTD which is either unphosphorylated or partially phosphorylated, as the IIa antibody could recognize both forms¹². To distinguish between these possibilities we did a sequential immunoprecipitation experiment. Supernatants derived from precipitations with the IIo antibody (Fig. 1b, lane 12) were re-immunoprecipitated with the IIo antibody (lane 13) (to ensure the sample was depleted of both the hyper- and partially phosphorylated forms of the CTD), and this second supernatant was then immunoprecipitated with the IIa antibody (lane 14). The resulting precipitate should contain polymerases with a completely unphosphorylated CTD. This third precipitate contains little *hsp70* DNA (lane 14), indicating that few elongating polymerases have a completely unphosphorylated CTD. A similar distribution of polymerase was found on the induced *hsp83* gene. These crosslinking results agree with a lower resolution survey of the distribution of RNA polymerase by immunofluorescence staining of polytene chromosomes, where it was found that the induced heat-shock puffs stained with both the IIo and IIa antibodies¹². Furthermore, our sequential precipitation of crosslinked complexes demonstrates that most of the elongating polymerases on the fully induced *hsp70* (and *hsp83*) gene contain a CTD that is either partially or hyper-phosphorylated.

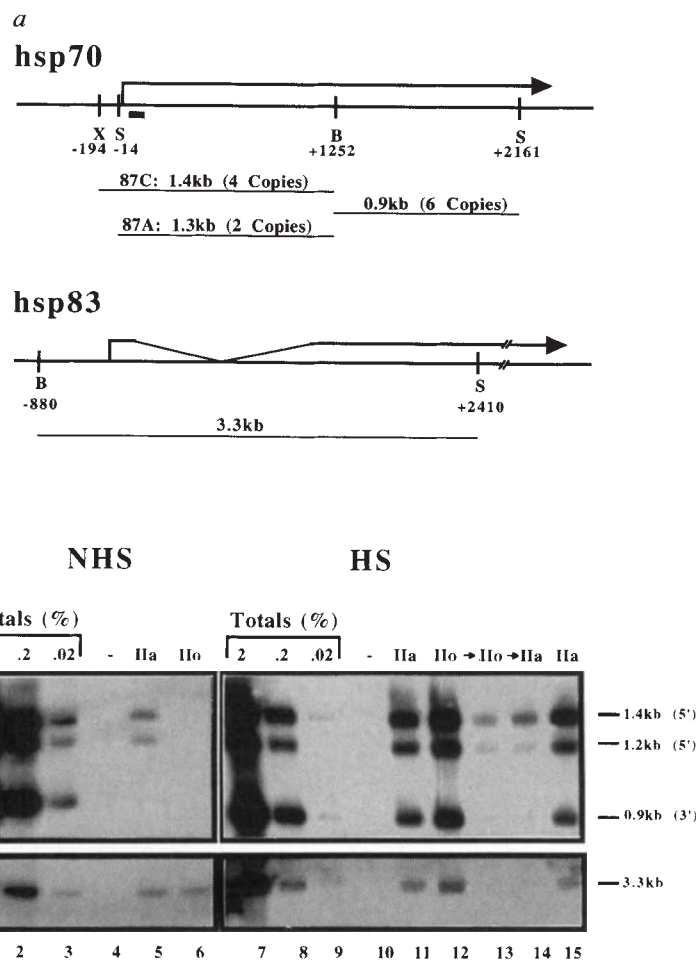
The partial phosphorylation of the CTD is not a consequence of the high rate of *hsp70* gene transcription. Cells that are heat shocked at an intermediate temperature (30 °C) produce a lower density of elongating polymerases (about 1 per gene, compared with about 30 per fully induced gene¹³). Under these conditions, both hyper- and partially phosphorylated RNA polymerases are found on the *hsp70* gene (Fig. 2, lanes 5 and 6), and the ratio on the 3' fragment is similar to that seen on the fully induced gene. Therefore, the level of CTD phosphorylation is not directly related to the frequency of transcription, at least in this range.

The uninduced *hsp26* gene also contains a paused polymerase, and by our crosslinking assay we find that it too is unphosphorylated (Fig. 3a). Furthermore, the 5'-most fragments of the constitutively expressed β -1 tubulin and *Gapdh-2* genes, which also contain a 5' paused polymerase^{7,10}, are immunoprecipitated more efficiently by the IIa than by the IIo antibody, in contrast to more 3' fragments (Fig. 3b, lanes 5 and 6), consistent with these paused polymerases being unphosphorylated. The histone *H1* gene does not have a 5' paused polymerase^{7,10}, and in this case, the IIa and IIo antibodies precipitate both the 5' and 3' fragments with equal efficiency (Fig. 3c, lanes 5 and 6). A similar ratio of IIa/IIo forms of polymerase is seen on the core histone genes (Fig. 3c). For the histone and *Gapdh-2* genes, elongating polymerases are recognized better by the IIa antibody than by the IIo antibody, suggesting that on these genes elongating polymerases are not as extensively phosphorylated as seen for

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FIG. 1 Distribution of the unphosphorylated and phosphorylated forms of RNA polymerase II *in vivo*. *a*, Restriction maps. *Drosophila* Kc cells contain 6 copies of the *hsp70* gene. The small filled bar at the 5' end of the *hsp70* gene indicates the region of polymerase pausing (from $\sim +20$ to $+40$)⁷⁻⁹. The *hsp83* gene also contains a 5' paused polymerase²⁰, but has not been mapped at high resolution. Restriction enzyme sites are: *Xho*I, X; *Bam*HI, B; *Sall*, S. *b*, Autoradiograms of filters containing restriction enzyme digested, immunoprecipitated DNA from non-heat shock (NHS) and heat shock (HS) cells probed to detect the *hsp70* gene (upper panel), and the *hsp83* gene (lower panel). A portion of the total DNA present in each immunoprecipitation was also examined (Totals; lanes 1-3, 7-9). Immunoprecipitations were carried out either with no antibody (-) (lanes 4 and 10), or with antibody directed against the unphosphorylated (IIa) (lanes 5 and 11), or phosphorylated form of the CTD (IIo) (lanes 6 and 12)¹², or sequentially with both antibodies (lanes 12-14). In lane 15, a sample was mock incubated with no antibody for the first two immunoprecipitations and then immunoprecipitated with the IIa antibody. METHODS. For the non-heat-shock and heat-shock samples each lane contains DNA isolated from 6×10^7 or 3×10^7 cells, respectively. Kc cells were either untreated or heat-shocked for 25 min at 37 °C, ultraviolet-treated, and the DNA and protein-DNA complexes purified as described¹¹ with the following modifications. The cell resuspension buffer was supplemented with 10 mM β -glycerophosphate (Sigma), 20 mM sodium fluoride (Sigma), and 1 mM sodium azide (Sigma). 1 mg ml⁻¹ Microcystin-LR (Sigma) was added to the nuclei lysis buffer. The dialysis buffer was also supplemented with 5 mM β -glycerophosphate. The antibodies were affinity purified as described¹². Each batch of affinity-purified antibody was individually titred to ensure that the antibody was added in excess of epitope. The probe used to detect polymerase on the *hsp70* gene was as previously described²⁰, and for *hsp83*, a *Bam*HI-*Sall* fragment spanning from -880 to +2,410²¹.



the β -1 tubulin or the fully induced *hsp70* or *hsp83* genes. Therefore, elongating polymerases appear to be phosphorylated to different levels in a gene-specific manner.

The paused polymerases at the 5' ends of many *Drosophila* genes contain a CTD which is predominantly unphosphorylated. Extensive phosphorylation of the CTD does not appear to be essential for transcription initiation or elongation to the pause, although we are unable to rule out the possibility that on initiation of transcription the CTD is phosphorylated and is dephosphorylated while paused. *In vitro*, the unphosphorylated form

of the CTD preferentially enters into the preinitiation complex^{14,16} and is the form that binds to the TATA binding protein (TBP)¹⁷. Like the polymerase that enters the preinitiation complex, the paused polymerase is unphosphorylated and therefore potentially capable of interacting with TBP. The CTD may continue to interact with TBP after initiation of transcription, acting as a flexible tether pausing polymerase over a region 20-40 bases downstream of the site of transcript initiation. The CTD also interacts with the SRB proteins^{18,19}, and these interactions may contribute to tether the paused polymerase. Alterna-

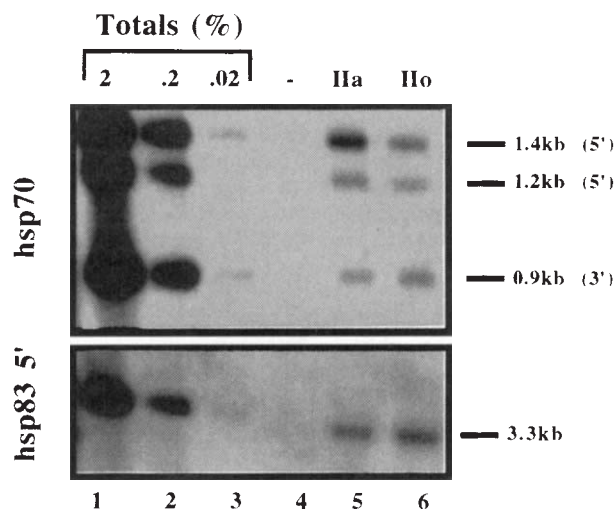


FIG. 2 Distribution of the IIa and IIo forms of RNA polymerase II on the *hsp70* gene at an intermediate level of heat shock (30 °C). See Fig. 1 legend for details. The 5' fragments are immunoprecipitated more efficiently by the IIa than by the IIo antibody. This is probably due to the lack of phosphorylation of an RNA polymerase that is known to be paused at the 5' end of each *hsp70* promoter during these intermediate heat shocks¹³.

METHODS. *Drosophila* Kc cells were heat-shocked at 30 °C for 25 min as described in the legend to Fig. 1. Protein-DNA complexes were digested with *Bam*HI, *Sall* and *Xho*I. Each lane contains DNA isolated from 6×10^7 cells. The probes used are described in Fig. 1.

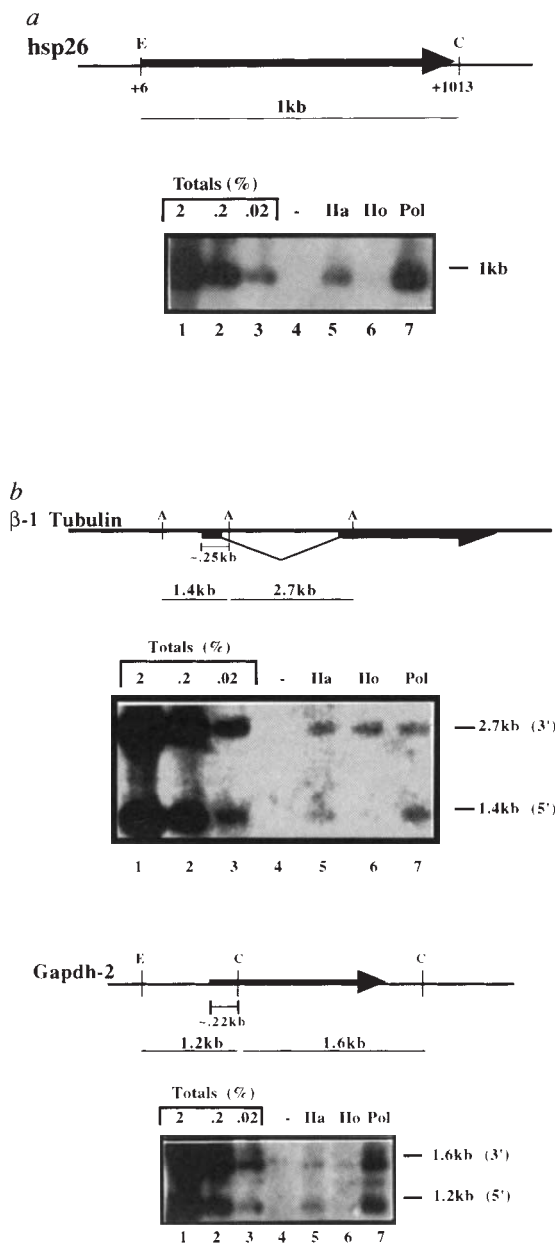
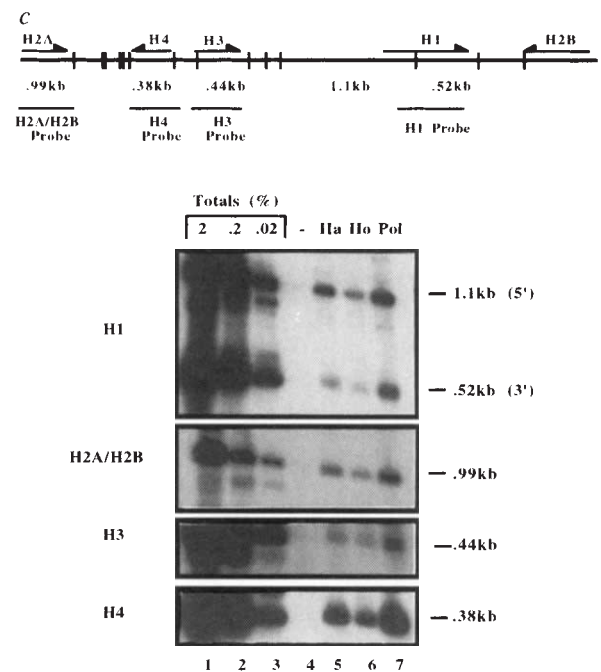


FIG. 3 Distribution of the Ila and Ilo forms of RNA polymerase II on various genes. **a**, *hsp26*. The paused polymerase is located between +23 and +56^{7,8}. *In vivo* ultraviolet crosslinking has shown that the only polymerase on the uninduced *hsp26* gene is the paused polymerase¹⁰. Immunoprecipitation using an antibody directed against the large subunit of RNA polymerase II is included (lane 7, Pol). **b**, β -1 tubulin and *Gapdh-2* genes. Exons 1 and 2 of the β -1 tubulin gene are represented by the filled arrow. The paused polymerase is located in the region +13 to +53⁷. The paused polymerase on the *Gapdh-2* gene has not been mapped at high resolution, but is located before +220¹⁰. **c**, Histone genes. The predicted fragments after *RsaI* digestion are shown. The histone genes are located on a 4.8-kb fragment that is repeated about 100 times²². The same filter was stripped and reprobed to detect polymerase density on the different histone genes. The ratio of immunoprecipitation by the Ila and Ilo antibodies (lanes 5 and 6) was quantified to be 2.0 for the H1 5' fragment, and 1.9 for the H1 3' fragment. **METHODS.** Each lane contains protein-DNA complexes isolated from 1×10^8 (β -1 tubulin gene) or 6×10^7 (*Gapdh-2* and histone genes) cells. Restriction enzyme sites are: *EcoRI*, E; *ClaI*, C; and *Avall*, A. The probes to detect polymerase on the β -1 tubulin, *Gapdh-2* and *hsp26* genes are as described^{10,20}. The probes used to detect polymerase on the histone genes are fragments isolated from pUH1.9 (H1)²³, pH2A (H2A/H2B)¹³, and pH4 (H4)¹³. The antibody against the large subunit of RNA polymerase II was described previously²⁴.



tively, the CTD of the paused polymerase may have severed its interactions with the basal factors, and the phosphorylation may be required to stimulate the paused polymerase to become elongationally competent. From our results, it would appear that phosphorylation of the CTD could either regulate the transition of polymerase from a paused to an elongating complex or be a consequence of this transition. □

Received 11 April; accepted 27 May 1994.

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ACKNOWLEDGEMENTS. We thank C. Giardina, X. Hua and J. Roberts for reading this manuscript. This work was supported by NIH grants to A.G. and J.T.L.

No limits on restriction

The structure of the endonuclease R.PvuII in the absence and presence of its recognition site indicates how the interaction with DNA might proceed.

THE bacterial restriction-modification (R-M) proteins did not evolve just for the convenience of molecular biologists and the profit of biotechnology companies, but to promote the survival of bacteria by helping to shape their primitive 'immune' systems. The sheer diversity of the sequence-specific type-II endonucleases that form one arm of the R-M system is also fascinating to students of biomolecular architecture, providing as they surely will a wealth of information on the nature of the interface between protein and DNA. Now two new structures are reported, one in this month's *Nature Structural Biology*¹ and the other to appear shortly in the *EMBO Journal*², of the PvuII endonuclease (R.PvuII) of the bacterium *Proteus vulgaris*. In the first¹ no DNA is present, but in the second the nuclease is complexed with its cognate DNA sequence (5'-CAGCTG-3')². The structures suggest a scenario for the initial steps of the protein-DNA interaction, as well as providing some clues about the general mechanism of action of the type-II restriction endonucleases.

Type-II bacterial R-M systems consist of a pair of proteins, both of which bind specifically to the same short, 4–8-base-pair recognition sequence. One of the pair is an endonuclease which, when bound to DNA in the presence of Mg²⁺, cleaves both strands of the DNA, cutting the duplex clean in two. The other is a DNA methyltransferase that adds a methyl group onto one of the bases in the recognition sequence. Methylation prevents binding of the recognition sequence by the endonuclease and hence the cleavage reaction. In this way the host bacterium's DNA is protected from destruction while foreign, unmethylated DNA — from viruses and other species of bacteria — is open to attack.

Over 2,000 restriction endonucleases, clocking up around 200 different DNA specificities between them, have been

isolated to date. The lack of homology between the 60 or so known gene sequences hints at the structural diversity of this class of enzymes. R.EcoRI and R.EcoRV show little similarity in either sequence, structure or mechanism of binding to DNA^{3,4}. On the other hand, R.BamHI does share substantial homology with R.EcoRI (refs 5, 6).

The structure of the unbound form of R.PvuII reveals that two monomers are non-covalently linked through a dimerization subdomain to form a U-shaped dimer¹; like many other restriction enzymes, R.PvuII is only active as a homodimer. The monomers are joined at the base of the 'U' by two helix-loop-helix structures — one from each monomer — which together form a pseudo three-helix bundle: an extensive hydrophobic core and a number of salt bridges and hydrogen bonds lock the bundle and the dimer together.

The shape of the dimer immediately suggests a possible binding site for DNA, neatly cradled in the cleft of the 'U'. Comparison of the R.PvuII structure with that of R.EcoRV bound to DNA³ further strengthens this suspicion. R.PvuII shares some structural and topological features with R.EcoRV, a restriction enzyme that, like R.PvuII, slices through the DNA duplex, leaving blunt rather than 5' or 3' overhanging ends. Elements of secondary structure that correspond to the DNA-binding elements of R.EcoRV can readily be identified in the structure of R.PvuII. The region that is similar to the 'R-turn' in R.EcoRV, which is involved in making base-specific contacts with DNA in this latter enzyme, falls across two antiparallel β -strands which line the inside of the cleft in apo-R.PvuII. Indeed, the co-crystal structure² reveals that this two-stranded sheet interacts with the major groove of the DNA in a fashion reminiscent of that seen for the MetJ and Arc repressors⁷. Similarly, the loop between the first and second helices of the dimerization subdomain is shown to be the equivalent of the R.EcoRV DNA-binding 'Q-turn', and again is situated strategically at the base of the cleft. This loop is seen to make backbone and base-specific contacts in the R.PvuII-DNA complex².

The residues and structures involved in catalysis in R.EcoRV have their counterparts in R.PvuII. But, unlike the DNA-binding domain, here the comparison can be extended to the catalytic residues of

R.EcoRI and R.BamHI (refs 5, 6) as well, for although these enzymes might interact with DNA in different ways, they all catalyse the cleavage of an identical DNA backbone to yield 5'-phosphate and 3'-hydroxyl groups. Whether this similarity is the result of convergent or divergent evolution is difficult to say — indeed the evolutionary relationship of the group as a whole is still a matter for debate⁵.

Although there are notable similarities between R.PvuII and R.EcoRV, there are also some important differences between the two structures. The cleft of the 'U' in the unbound form of R.PvuII is much wider than it is in the structure of both free and bound R.EcoRV and so could not contact DNA as extensively with both subunits as in R.EcoRV. Athanasiadis and colleagues¹ suggest that the initial stages of the protein-DNA interaction may occur through the equivalent of the R.EcoRV Q-turn. In the unbound form, this loop in the two monomers would be the only DNA-binding motif capable of making symmetry-related contacts with the DNA recognition site. The potential flexibility of the loops connecting the various subdomains would then allow the enzyme to grasp the DNA more tightly. Indeed, the co-crystal shows a dramatic conformation change in R.PvuII: the mouth of the cleft closes up, wrapping around the DNA and bringing the tips of the two arms of the 'U' into contact, a movement of some 25 Å. The DNA, on the other hand, does not deviate significantly from B-form, indicating that in this case distortion of the double helix is not required for recognition².

We can look forward to the next clutch of structures furnished by the restriction endonucleases, which no doubt will continue to provide a pertinent illustration of the variability and unpredictability of the protein-DNA interface.

Guy Riddihough

Guy Riddihough is Editor of Nature Structural Biology.

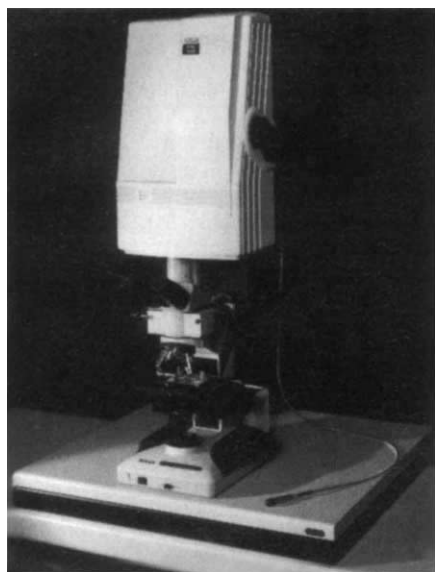
Also in this month's *Nature Structural Biology*: a copper-iron-sulphur cluster in a ferredoxin; apomyoglobin as a molten globule; haem binding in a bacterioferritin; the unique structure of *Serratia* endonuclease; GroEL fails to recognize a molten globule; the general base in ras p21; and entropic effects of disulphide bonds on protein stability.

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6. Newman, M., Strzelecka, T., Dörner, L.F., Schildkraut, I. & Aggarwal, A.K. *Structure* **2**, 439–452 (1994).
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A sharper image

New in the microscopy/image analysis marketplace — a transmission electron microscope for the non-specialist, an advanced fluorescent *in situ* hybridization system and an upgraded version of software for intracellular ion imaging.

In the confocal microscopy line Bio-Rad offers the MRC-1000 **confocal laser scanning imaging system** — a computer-controlled, multichannel laser scanning imaging system with electronics that have been



Bio-Rad's new confocal configuration.

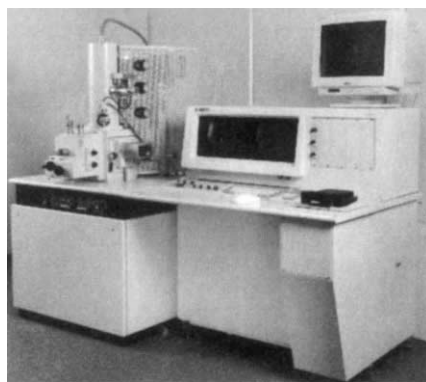
redesigned and relocated to enhance the signal-to-noise ratio (*Reader Service No. 100*). All the main features in the MRC-1000 system are under computer control. The system is offered with up to three independent confocal detection channels, each with computer controlled gain and black level. The confocal iris and six-position emission filter wheel on each channel are also independently controlled by the computer. Set up of the instrument for a particular fluorochrome can be preset as a method and recalled by a single key stroke. The system offers simultaneous acquisition of multiple channel data and signal mixing electronics for combined display of multichannel data. The capability for subtractive mixing is intended to provide the facility for real-time, bleed-through corrections. Visible wavelength and ultraviolet configurations are available.

■ TEMs/SEMs

The new EM 208 **transmission electron microscope** from Philips, designed with the needs of the non-specialist in mind, is intended for use in routine analysis in the life sciences area — such as anatomy, histology, pathology and virology (*Reader Service No. 101*). Billed as a successor to the company's EM 201 TEM, the EM 208 shares the same high-power objective lens, which is designed to provide high-contrast images even in high ambient lighting conditions, and has a mag-

nification range of $\times 25$ to $\times 200,000$. The emphasis is on ease of use: only five controls are required from insertion of the specimen to recording of the image, and all of the main operating parameters — such as alignment of the column, stigmator and vacuum — are computer controlled. Vacuum in the EM 208 is maintained using a rotary pump and an oil diffusion pump; a buffer tank eliminates the need for the pump to run continuously. In addition, the system uses tungsten sources. Image contrast can be varied by altering the accelerating voltage in 10-kV steps. Besides the standard version with a fluorescent screen, the system is also available with a TV display. The TV version is capable of printing out images on a video printer (a feature intended to cut running costs by eliminating the need for a darkroom and photographic development).

Hitachi says that its new S-4200 **field emission scanning electron microscope** provides a resolution of better than 5 nm at 1 kV and 1.5 nm at 15 kV, and is suitable for high-resolution studies of beam-sensitive materials and biological specimens (*Reader*



Hitachi's field emission SEM.

Service No. 102). The instrument, which incorporates the company's long-life, cold field emission electron gun, features auto-column alignment, a choice of two stages, and a large chamber that can accommodate specimens up to 150 mm in diameter. Two high-resolution framestores provide opportunities for recording, retrieving and comparing images. Their storage capacity can be expanded further through the addition of an optional Magneto Optical disk unit, or other external archiving systems. Hitachi says that the S-4200 is especially suited to qualitative and quantitative

microanalytical studies because it allows a high X-ray takeoff angle of 30° . It is also suitable for cryo-studies. Optional accessories are available for cathodoluminescence, back-scattered electron detection, secondary electron detection, heating of the specimen and tensile stages.

■ Workstations

The latest version of the SmartCapture advanced **fluorescent *in situ* hybridization system** (FISH system) from Imagenetics includes new tools to optimize the clarity and detail of hybridization results obtained using the FISH technique (*Reader Service No. 103*). These new functions are designed to reduce or remove background noise so that faint signals can be identified, to enhance the details of the image, and to extend the utility of samples, such as those from comparative genomic 4',6-diamidino-2-phenylindole acid (DAPI). The workstation, which utilizes a cooled digital CCD camera (Photometrics) automatically controls the exposure, the filter wheel and the construction of a 24-bit colour image. Algorithms for determining colour ratios and for elucidating chromosome banding patterns from DAPI or propidium iodide counterstained slides are a few of the analytical functions available.

Bio Image's (Millipore) new Intelligent Quantifier **gel scanner system** is being offered for under \$20,000 (*Reader Service No. 104*). The 'IQ' system, which consists of a computer, a 1,200 d.p.i. scanner and Bio Image software, can be used to analyse a wide variety of electrophoresis gels and membranes. The software consists of a self-installing, self-tutoring program that supports whole-band, one-dimensional analysis, two-dimensional electrophoresis, blot analysis and colony/plaque counting. A user can annotate and compare images and prepare reports without leaving the keyboard using Microsoft Excel and Word, Lotus 123 and WordPerfect. The system can be networked for comparing and sharing data from multiple sources.



Bio Image's 'IQ' gel scanner system.

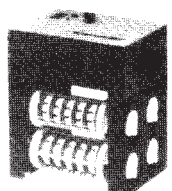
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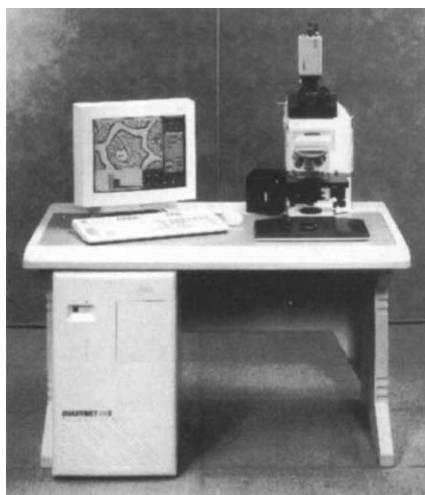
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Quantimet 600 is the new **image processing and analysis system** from Leica (*Reader Service No. 105*). If high definition is required, the system can handle up to $4,096 \times 4,096$ pixels per image. A morphological processor is used to enhance images and discard unwanted detail. The Quantimet 600 is operated from a graphical user interface using the QWin interactive image analysis



Leica's next generation analysis system.

software. Repeatable measurement routines are created with the built-in Quips image processing macro software. The Quantimet 600 is part of a family of image processing and analysis products that range from the entry-level Quantimet 500+ through to the standard TV resolution Quantimet 600S and the high-resolution HR version.

■ Software

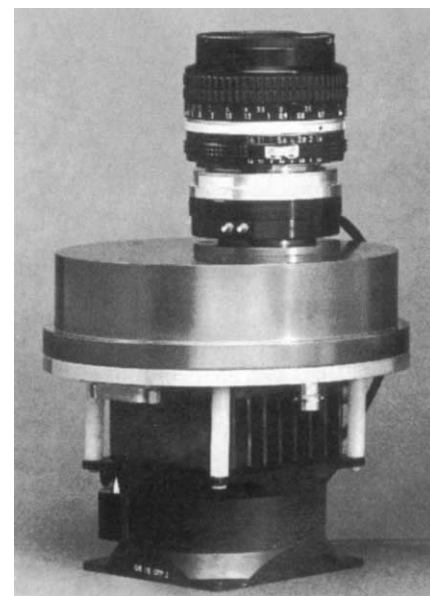
ImproVision has released a new version, 1.36, of its **ionVision intracellular ion imaging and analysis system software** (*Reader Service No. 106*). Three new features — online ratioing, electrophysiology synchronization and the automation of drug delivery — are included in the upgraded version. The first gives users the option of seeing a ratio image displayed at the same time as the camera image. The ratio image can be displayed even if the original image data are being stored to the hard or optical disk. The electrophysiology addition allows ionVision to synchronize the collection of images to event points on an external electrophysiology system. IonVision can act as either master or slave, thereby allowing full hand-shaking and the option of acquisition speed control for the external system. The drug delivery control provides for full automation of the experiment (the release of up to eight different solutions/drugs at predetermined time points can be preprogrammed).

Foster Findlay is offering an upgrade of its **PC_Image image analysis program** for Windows (*Reader Service No. 107*). Extensive data analysis capabilities are featured, and results can be exported to other Windows programs (such as spreadsheets) via

dynamic data exchange (DDE). The program is available for a wide range of framestores from most of the leading manufacturers, and there is also a version for users with pre-digitized images. A new macro facility enables the user to customize PC_Image, and some new measurement functions have been added. A DDE link to the imaging database 'Treasury' (Synoptics) has been incorporated.

■ Cameras

First Magnitude has introduced the **StarScape III high-performance CCD camera** (*Reader Service No. 108*). The camera uses the Loral/Fairchild CCD422 $1,024 \times 1,024$ array (15×15 micron pixels), and features very low dark current (MPP mode) and read-out noise of only 4 electrons rms (at 50 kHz), with optional readout rates up to 1 MHz. For researchers on a budget, the company has introduced a lower-cost StarScape II version, available with either the Texas Instruments TC215 $1,024 \times 1,024$ or the TC217 $1,138 \times 488$ CCD arrays (12×7.9 micron pixels), either liquid nitrogen or thermoelectrically cooled. These new systems come



StarScape CCD camera.

complete with data acquisition under Windows 3.1, including image acquisition and display, zoom, histogram, row and column plot, printing, adjustable stretch and an automatic testing mode. Also included is StarSoft 3.1 spectra acquisition and display software, which can be run along with third-party spectral reduction and analysis software such as SpectraCalc. Systems include an IBM PC 386/486 plug-in interface card and cables.

Seescan says that its **low-light camera** uses a noise reduction technique to give a similar sensitivity and image quality to that of cooled cameras and is also competitively priced (*Reader Service No. 109*). Stated applications include fluorescence microscopy and gel documentation. Operating modes

include live, single-shot and triggered image capture with timing facilities for controlling external devices like shutters and time-lapse video recorders. Several display modes are offered, including normal, enhanced contrast and pseudocolour. Hard copies can be produced by connecting to a postscript or video printer, and images may be transferred in digital format to IBM or compatible PCs or Macs.

Paultek Imaging has developed a **video rate CCD camera** designed for high spatial resolution, low-light-level detection and with features such as optional control of all operating modes and parameters via its RS-232 port (*Reader Service No. 110*). Specifications include 1,134 cells per line and 486 lines. The architecture of the two-thirds inch TI frame transfer sensor chip enables it to be operated in an extended (multi-frame) integration mode followed by the output of a single high-level frame containing two balanced interlaced fields compatible with RS-170 frame grabbers. Thermoelectric cooling is optional. It is also capable of capturing pulsed images. Both fields receive equal exposure and are output in standard interlaced RS-170 format. The camera includes a microcontroller which accepts inputs either from the control panel or the RS-232 port.

■ Peripherals

A new line of long-working distance **water immersion objectives** intended for electrophysiology, *in vivo* experiments, microcirculation and other applications in the life sciences where an upright microscope needs to be used, is offered by Carl Zeiss (*Reader Service No. 111*). The LD Achromplan ICS



Achromplan water immersion objectives.

Infinity Colour-Corrected System) water immersion objectives feature a slender objective barrel with a ceramic housing, designed to provide good access for micromanipulation and microinjection while, at the same time, maintaining electrical and thermal isolation for patch-clamp techniques. Suitable for use in brightfield, phase DIC and epifluorescence microscopy, the new objectives cover the range from $\times 10$ to $\times 63$.

The new TX-1100 **colour thermal printer** from Polaroid is designed for producing A-6 prints from a wide range of video sources in under a minute (*Reader Service No. 112*). The printer features new advances



Polaroid's colour thermal printer.

in thermal dye diffusion transfer technology and can receive and store PAL, S-VHS or RGB video images. Up to 25 copies of the same image or different images can be reproduced on each individual print, if desired. Borders can be cropped to reproduce only the desired portion of the image and any detail in the image can be enlarged up to 200 per cent. In addition, text can be printed directly onto prints for easy storage.

Developed specifically for DNA sequencing applications, the new BioMax MR **sequencing film** from Kodak features a single-emulsion format designed to increase resolution and sensitivity to all ^{35}S -, ^{33}P - and ^{14}C -labelled gels (*Reader Service No. 113*). For detection of ^{35}S , ^{33}P and ^{14}C , it is said to be twice as sensitive as the company's X-Omat AR(XAR) film. Because it is coated with emulsion on only one side, Kodak says the film also provides maximum resolution and clarity for detection of ^{32}P and chemiluminescence labels, although the company says that its X-Omat AR film is twice as sensitive as BioMax MR film for detection of ^{32}P and ^{125}I .



BioMax film.

The EMS 820 **laboratory microwave oven** available from Electron Microscopy Sciences is intended for use in the many steps in sample preparation carried out prior to either light or electron microscopy, including — but not limited to — staining, fixation, decalcification, impregnation, dehydration and immunohistochemistry (*Reader Service No. 114*). Design features include a pushbutton timing mechanism, a continuous run control button for longer procedures over 99 min/99 s, a mixing arrangement for up to five different containers at once, a stainless steel compartment and an adjustable temperature probe.

■ Cell/tissue preparation

The Krumdieck **tissue slicer** from Alabama Research and Development is an automated microtome which can be sterilized and which is designed for the preparation of aseptic slices of live tissues for organ culture (*Reader*

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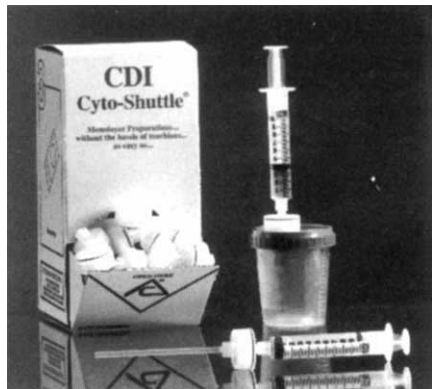
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PRODUCT REVIEW

Service No. 115). The device is designed for making reproducible slices (as thin as 60 microns) with evenly cut surfaces. Stated applications include biochemistry, immunology, neurology, pharmacology, physiology and toxicology.

Cyto-Shuttle is a disposable device available from Cancer Diagnostics for **monolayer slide preparation** said to combine the advantages of filter cytology without the presence of filter artifacts (*Reader Service No. 116*). As part of the procedure, the filter is removed, leaving the sample ready for staining and examination. The device is designed



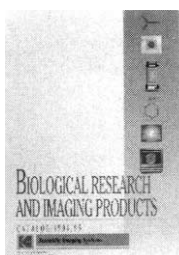
CDI's cytology monolayer apparatus.

to optimize routine cytological applications, cytogenetics, haematology, urology and microbiological applications. Monolayer preparations prepared in this way should, says CDI, be particularly useful in image analysis, immunocytochemistry, *in situ* hybridization and FISH. When used with the Cyto-Straw, a disposable plastic tube that attaches to the Cyto-Shuttle, samples can be drawn directly into the Cyto-Shuttle from any container (even a 50-ml tube), and is especially useful for small samples. Replaceable filters, allowing multiple slide preparation from one Cyto-Shuttle, should make it more cost-effective.

■ Catalogues and services

Advanced Surface Microscopy, an **independent testing laboratory** dedicated to providing a range of analytical services, has now improved its analytical capabilities with the installation of a NanoScope Dimension 3000 scanning probe microscope (*Reader Service No. 117*). The Dimension 3000 supports a wide range of scanning probe techniques. In addition to imaging topography and measuring roughness on both hard and soft surfaces, it senses elasticity, friction and magnetic and electric forces. Large objects, such as 8-inch diameter silicon wafers, magnetic media and optical disks, are placed

directly in the microscope without the need for cutting or other preparation.



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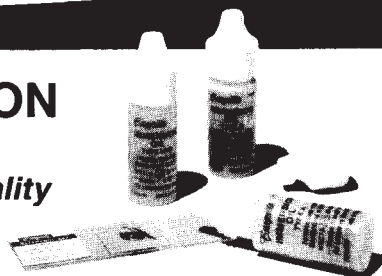
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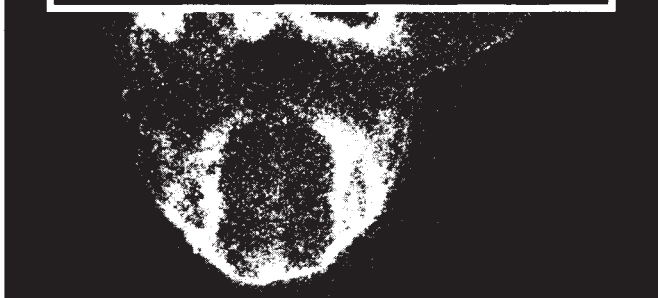
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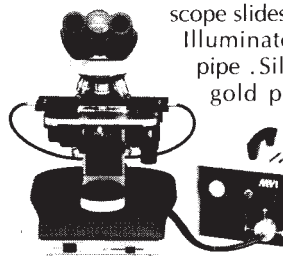
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